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(54) **Conjugates of methyltrithio antitumor agents and intermediates for their synthesis**

Konjugate von Methyltrithio-Antitumormitteln und Zwischenprodukte für deren Herstellung

Conjugaison d'agents méthyltrithio antitumoraux et leurs intermédiaires de synthése

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- **BERNSTEIN, I .D. ET AL.: "Treatment of acute myeloid leukemia cells in vitro with a monoclonal antibody recognizing a myeloid differentation antigen" J. CLIN. INVEST., vol. 79, 1987, page 1153 XP002106979**

EP 0 689 845 B1

Description

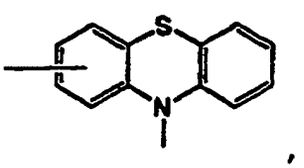
SUMMARY OF THE INVENTION

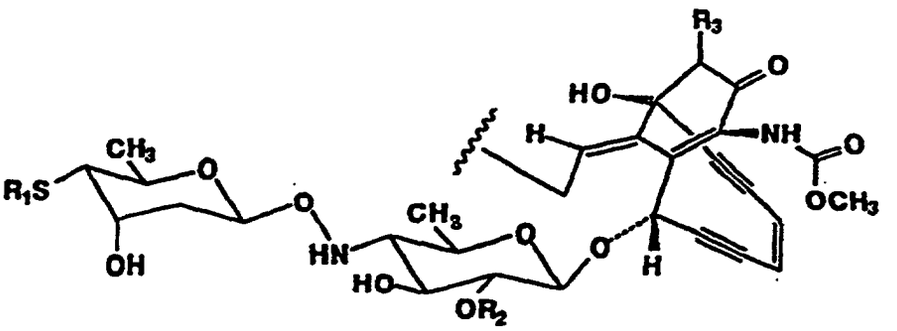
[0001] This invention describes carrier-drug conjugates prepared from disulfide analogs of the calicheamicin family of potent antitumor antibiotics and their derivatives, as well as similar analogs from related antitumor antibiotics such as the esperamicins. The carrier can be an antibody, growth factor, or steroid which targets an undesired population of cells, such as those of a tumor. Whole protein carriers as well as their antigen-recognizing fragments and their chemically or genetically manipulated counterparts are useful for the targeting portion of the conjugates. This invention includes compounds required for the synthesis of these conjugates, appropriate pharmaceutical compositions of the carrier-drug conjugates, and their method of use.

[0002] More specifically, one aspect of the invention includes a cytotoxic drug conjugate of the formula:

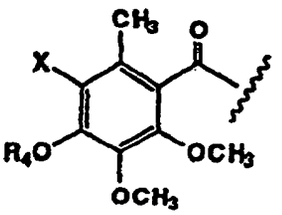
$$Z^3[CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=Z^2]_m$$

wherein

Z^3	is a protein selected from mono- and polyclonal antibodies, their antigen-recognizing fragments, and their chemically or genetically manipulated counterparts and growth factors and their chemically or genetically manipulated counterparts, wherein a covalent bond to the protein is an amide formed from reaction with "m" lysine side chains, or a steroid, wherein the covalent bond to the steroid is an amide or an ester;
m	is from about 0.1 to 15;
Alk^1 and Alk^2	are independently a bond or branched or unbranched (C_1-C_{10}) alkylene chain;
Sp^1	is a bond, -S-, -O-, -CONH-, -NHCO-, -NR'-, -N(CH_2CH_2)$_2$N-, or -X-Ar'-Y-(CH_2)$_n$-Z wherein X, Y, and Z are independently a bond, -NR'-, -S-, or -O-, with the proviso that when n = 0, then at least one of Y and Z must be a bond and Ar' is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, COOR', CONHR', O(CH_2)$_n$COOR', S(CH_2)$_n$COOR', O(CH_2)$_n$CONHR', or S(CH_2)$_n$CONHR', with the proviso that when Alk^1 is a bond, Sp^1 is also a bond;
n	is an integer from 0 to 5;
R'	is a branched or unbranched (C_1-C_5) chain optionally substituted by one or two groups of -OH, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, (C_1-C_3) dialkylamino, or (C_1-C_3) trialkylammonium-A^- where A^- is a pharmaceutically acceptable anion completing a salt;
Sp^2	is a bond, -S-, or -O-, with the proviso that when Alk^2 is a bond, Sp^2 is also a bond;
Z^1	is H, (C_1-C_5) alkyl, or phenyl optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, COOR', CONHR', O(CH_2)$_n$COOR', S(CH_2)$_n$COOR', O(CH_2)$_n$CONHR', or S(CH_2)$_n$CONHR' wherein n and R' are as hereinbefore defined;
Ar	is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, COOR', CONHR', O(CH_2)$_n$COOR', S(CH_2)$_n$COOR', O(CH_2)$_n$CONHR', or S(CH_2)$_n$CONHR' wherein n and R' are as hereinbefore defined or a 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6-, or 2,7-naphthylidene or  , each naphthylidene or phenothiazine optionally substituted with one, two, three, or four groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, or COOR', CONHR', O(CH_2)$_n$COOR', S(CH_2)$_n$COOR', O(CH_2)$_n$CONHR', or S(CH_2)$_n$CONHR' wherein n and R' are as hereinbefore defined, with the proviso that when Ar is naphthylidene, Z^1 is not hydrogen and with the proviso that when Ar is phenothiazine, Sp^1 is a bond only connected to nitrogen;
Z^2	is Q-Sp-S-S-W, wherein W is

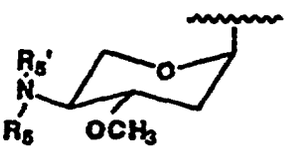


R_1 is



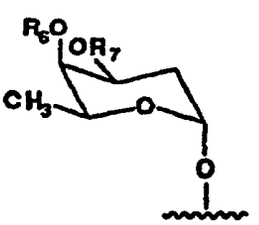
or CH_3;

R_2 is



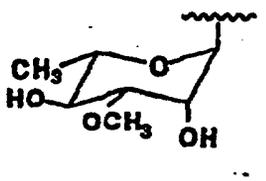
or H;

R_3 is



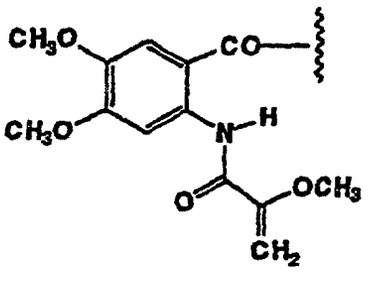
or H;

R_4 is



or H;

R_6 or R_7 is H or



;

R_5 is $-CH_3$, $-C_2H_5$, or $-CH(CH_3)_2$; X is an iodine or bromine atom; $R_{5'}$ is a hydrogen or the group RCO, wherein R is hydrogen, branched or unbranched (C_1-C_{10}) alkyl or (C_1-C_{10}) alkylene group, a (C_6-C_{11}) aryl group, a (C_6-C_{11}) aryl-alkyl (C_1-C_5) group, or a heteroaryl or heteroaryl-alkyl (C_1-C_5) group wherein heteroaryl is defined as 2- or 3-furyl, 2- or 3-thienyl, 2- or 3-(N-methylpyrrolyl), 2-, 3-, or 4-pyridyl, 2-, 4-, or 5-(N-methylimidazolyl), 2-, 4-, or 5-oxazolyl, 2-, 3-, 5-, or 6-pyrimidinyl, 2-, 3-, 4-, 5-, 6-, 7-, or 8-quinolyl, or 1-, 3-, 4-, 5-, 6-, 7-, or 8-isoquinolyl, all aryl and heteroaryl groups optionally substituted by one or more hydroxy, amino, carboxy, halo, nitro, lower (C_1-C_3) alkoxy, or lower (C_1-C_5) thioalkoxy groups;

Sp is a straight or branched-chain divalent or trivalent (C_1-C_{18}) radical, divalent or trivalent aryl or heteroaryl radical, divalent or trivalent (C_3-C_{18}) cycloalkyl or heterocycloalkyl radical, divalent or trivalent aryl- or heteroaryl-alkyl (C_1-C_{18}) radical, divalent or trivalent cycloalkyl- or heterocycloalkyl-alkyl (C_1-C_{18}) radical or divalent or trivalent (C_2-C_{18}) unsaturated alkyl radical, wherein heteroaryl is furyl, thienyl, N-methylpyrrolyl, pyridinyl, N-methylimidazolyl, oxazolyl, pyrimidinyl, quinolyl, isoquinolyl, N-methylcarbazoyl, aminocoumarinyl, or phenazinyl and wherein if Sp is a trivalent radical, it can be additionally substituted by lower (C_1-C_5) dialkylamino, lower (C_1-C_5) alkoxy, hydroxy, or lower (C_1-C_5) alkylthio groups; and

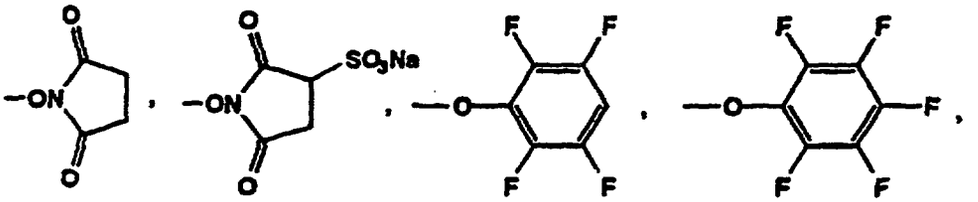
Q is =NHNCO-, =NHNCS-, =NHNCONH-, =NHNCSNH-, or =NO- and includes the conjugates use as antitumor agents.

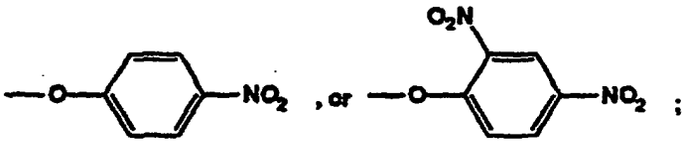
[0003] A second aspect of this invention involves modified drugs, useful as intermediates for constructing conjugates, of the formula:

$$Z^3\ [CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=Z^2]_m$$

wherein Z^1, Z^2, Alk^1, Sp^1, Ar, Sp^2, and Alk^2 are as hereinbefore defined;

Z^3 is halogen, hydroxy, OM wherein M is a metal completing a salt, $-N_3$,





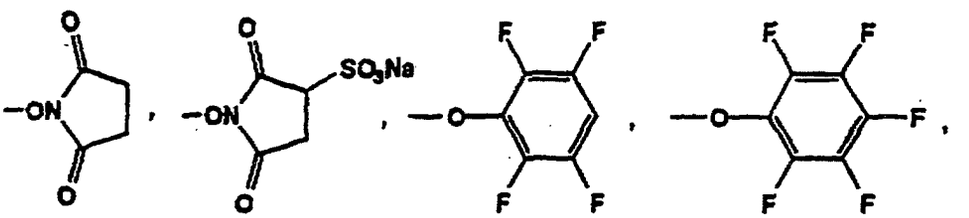
and m is 1.

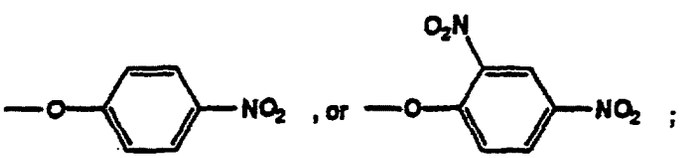
[0004] A third aspect of this invention involves linkers, useful for constructing drug conjugates, of the formula:

$$Z^3\ [CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=Z^2]_m$$

wherein

Z^3 is halogen, hydroxy, OM wherein M is a metal completing a salt, $-N_3$,





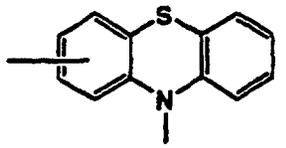
Alk^1 and Alk^2 are independently a bond or branched or unbranched (C_1-C_{10}) alkylene chain;

Sp^1 is a bond, -S-, -O-, -CONH-, -NHCO-, -NR'-, $-N(CH_2CH_2)_2N-$, or -X-Ar'-Y-$(CH_2)_n$-Z wherein X, Y, and Z are independently a bond, -NR'-, -S-, or -O-, with the proviso that when n = 0, then at least one of Y and Z must be a bond and Ar' is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$, with the proviso that when Alk^1 is a bond, Sp^1 is a bond;

n is an integer from 0 to 5;

R' is a branched or unbranched (C_1-C_5) chain optionally substituted by one or two groups of -OH, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, (C_1-C_3) dialkylamino, or (C_1-C_3) trialkylammonium - A- where A^- is a pharmaceutically acceptable anion completing a salt;

Ar is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, or COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCO$-OR', $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ wherein n and R' are as hereinbefore defined or a 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6-, or 2,7-naphthylidene or



each naphthylidene or phenothiazine optionally substituted with one, two, three, or four groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, or COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ wherein n and R' are as hereinbefore defined, with the proviso that when Ar is naphthylidene, Z^1 is not hydrogen and with the proviso that when Ar in phenothiazine, Sp^1 is a bond only connected to nitrogen;

Sp^2 is a bond, -S-, or -O-, with the proviso that when Alk^2 is a bond, Sp^2 is a bond;

Z^1 is H, (C_1-C_5) alkyl, or phenyl optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ wherein n and R' are as hereinbefore defined;

Z^2 is oxygen; and

m is 1,

with the proviso that when Ar is unsubstituted 2/6-naphthylene or 1,3- or 1,4-phenylene optionally substituted with one group of (C_1-C_6) alkyl or (C_1-C_5) alkoxy and Alk^2 is a bond, then Sp^1 is not a bond, -O-, or -NHCO-.

DESCRIPTION OF THE DRAWINGS

[0005] Chart 1: DNA and amino acid sequences for h-P67.6 light chain.

[0006] Chart 2: DNA and amino acid sequences for h-P67.6 heavy chain.

[0007] Chart 3: Plasmid for h-P67.6 heavy chain expression.

[0008] Chart 4: Plasmid for insertion of h-P67.6 heavy chain.

[0009] Chart 5: Plasmid for h-P67.6 light chain expression.

[0010] Fig. I: The proton magnetic resonance spectrum of 4-formylphenoxyacetic acid condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

[0011] Fig. II: The proton magnetic resonance spectrum of 4-formylbenzoic acid condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

[0012] Fig. III: The proton magnetic resonance spectrum of 4-formyl-3-methoxyphenoxyacetic acid condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

[0013] Fig. IV: The proton magnetic resonance spectrum of 6-formyl-2-naphthoic acid condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

[0014] Fig. V. The proton magnetic resonance spectrum of 4-(4-acetylphenoxy)butanoic acid condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

[0015] Fig. VI: The proton magnetic resonance spectrum of 4-(4-acetylphenoxy)butanoic acid condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccinimide ester.

[0016] Fig. VII: The proton magnetic resonance spectrum of 4-(4-formylphenoxy)butanoic acid condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

BACKGROUND OF THE INVENTION

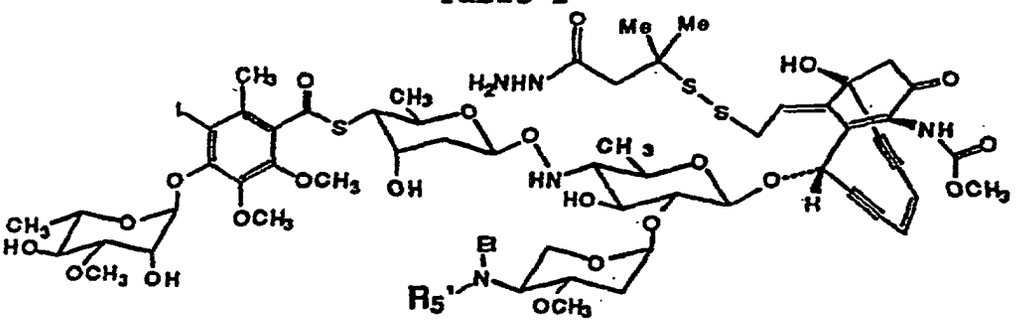
[0017] Since the discovery of methodology for producing monoclonal antibodies was published in the 1970's (G. Köhler and C. Milstein, "Nature" **256**, 495 (1975)), numerous attempts have been made to use these proteins to achieve selective targeting of antitumor agents to tumors. (E.g., see T. Ghose and A. H. Blair, "CRC critical Rev. Drug Carrier Systems" **3**, 263 (1987), G. A. Koppel, "Bioconjugate Chem." **1**, 13 (1990), and J. Upeslacis and L. Hinman, "Ann. Rep. Med. Chem." **23**, 151 (1988).) Although progress continues to be made in this field, most classical antitumor agents produce antibody conjugates which are relatively ineffective for a variety of reasons. Among the reasons for this ineffectiveness is the lack of potency of the chemotherapeutic agent and its poor utilization due to the lack of efficient release of the drug at its site of action.

[0018] The potent family of antibacterial and antitumor agents, known collectively as the calicheamicins or the LL-E33288 complex, are described and claimed in U.S. Pat. No. 4,970,198 (1990). The most potent of the agents is

designated γ_1^I, which is herein referred to simply as gamma. The dihydro derivatives of these compounds are described in U.S. Pat. No. 5,037,651 (1991) and the N-acylated derivatives are described in U.S. Pat. No. 5,079,233 (1992). Related compounds which are also useful in this invention include the esperamicins which are described and claimed in U.S. Pat. No. 4,675,187 (1987); 4,539,203; 4,555,162 (sic U.S. Patent No. 4,554,162); and European Patent 289,030. All of these compounds contain a methyltrisulfide that can be reacted with appropriate thiols to form disulfides, at the same time introducing a functional group such as a hydrazide or similar nucleophile. Examples of this reaction with the calicheamicins are given in U.S. Pat. 5,053,394 which also discloses targeted forms of the calicheamicins.

[0019] Two compounds which are useful for the synthesis of conjugates with carrier molecules, as disclosed and claimed in U.S. Pat. No. 5,053,394, are shown in Table 1.

Table 1



$R_5' = H$ gamma dimethyl hydrazide

$R^{5'} = Ac$ N-acetyl gamma dimethyl hydrazide

[0020] Included as carrier molecules in U.S. Pat. No. 5,053,394 (see also EP 392384) are steroids, growth factors, antibodies, antibody fragments, and their genetically or enzymatically engineered counterparts, hereinafter referred to singularly or as a group as carrier. The essential property of the carrier is its ability to recognize an antigen or receptor associated with an undesired cell line. Examples of carriers are given in U.S. Pat. No. 5,053,394, and such carriers are also appropriate in the present invention. Antibody carriers can be from almost any mammalian species (eg. mouse, human, dog, etc.) and can be produced by various methods (eg. murine antibodies via hybridomas, human antibodies via hybridomas from transgenetic mice, etc).

[0021] Specific examples of carriers which are exemplified herein are the antibodies P67.6, A33, CT-M-01 and the "anti-Tac" antibody of Waldman. These antibodies are used here in two forms: a murine form, designated by an "m" (e.g., m-P67.6), and a genetically engineered, humanized form, designated by an "h" (e.g., h-P67.6) whenever appropriate. The basic technology for humanization is disclosed by Winter in US Patent 5,225,539 (1993) and by Adair in WO 91/09967 (1991). m-P67.6 is disclosed in I.D. Bernstein et al., "J. Clin. Invest." **79**, 1153 (1987) and recognizes the CD33 antigen which is prevalent on certain human myeloid tumors, especially acute non-lymphocytic leukemia (ANLL).

[0022] Chart 1 and Chart 2 show the DNA coding and predicted amino acid sequences of the variable regions of one particular h-P67.6 that is particularly suitable for use in the present invention. The framework for this antibody is the EU framework for human IgG_4 shown in Gottlieb et al., "Biochemistry: 9, 3115 (sic 3155) and 3161 (1970). The antibody was prepared using the general strategy described in WO 91/09967.

[0023] With reference to the charts, the overlapping oligonucleotides that were synthesized (Oligo L1 through L8) are shown with double underlines, and the PCR assembly procedure (cf. WO 92/01059), was applied to these sequences. The CDR regions of the protein are designated with single underlines and other amino acids that were taken from the murine sequences are shown with a double underline. The restriction sites that were used to splice the sequences into plasmids are indicated at the beginning and end of the sequences. The variable portion of the heavy chain was cloned into plasmid pMRR14 (WO 93/06231) to give the plasmid designated pAL63 (Chart 3) and the variable portion of the light chain was cloned into plasmid pMRR15 (Chart 4) to give pAL60 (Chart 5). Plasmids pMRR14 and pMRR15 contained the constant regions of the heavy and light chains. respectively, and therefore pAL63 and pAL60 contained complete sequences for the P67.6 heavy and light chains. The plasmids were cotransfected into CHO-L761 cells to generate a h-P67.6 producing line from which the h-P67.6 was purified by standard methods. The resultant h-P67.6 bound to HL60 cells in competition with murine antibody with about a 50% loss in immunoaffinity. This binding was inhibited by pre-incubation with soluble CD33 antigen.

[0024] The antibody m-CT-M-01 is disclosed in E.P. 86 401482.4/0208615 and recognizes the polyepithelial mucin

(PEM) antigen present on many human solid tumors, particularly breast, lung, and ovarian. The humanized version of this antibody, h-CT-M-01, is described in WO 93/06231 (1993). The antibody m-A33 is disclosed in US patent application serial numbers 327,765; 673,153; and 671,132 (now issued as US Patents Nos 5160723 and 5431897) and is a murine antibody which recognizes a glycoprotein antigen present on colon cancer cells. The humanized version of this antibody, h-A33, is disclosed in UK Patent Application 9,315,249.4.

[0025] Anti-Tac is disclosed in T. A. Waldman et al., "J. Immunol." **126,** 1393 (1981) and is a murine antibody reactive with the IL-2 receptor that is found on activated and functionally mature T cells, including abnormally activated leukemia cells.

[0026] The two basic types of conjugates disclosed in U.S. Pat. No. 5,053,394 are those which are attached to lysine residues of the antibody and those which are attached to the oxidized carbohydrate residues using the method taught in U.S. Pat. No. 4,671,958. Lysine attachment as it is disclosed in U.S. Pat No. 5,053,394 produces conjugates which are stable to hydrolysis under normal physiological conditions. The carbohydrate-based conjugates, which involve the formation of a hydrazone from a hydrazide or similar derivative, are hydrolytically unstable under certain conditions, and that is in many cases an advantage. Some instability is often needed to allow release of the drug once the conjugate has been internalized into the target cell, but a certain degree of stability is important to prevent premature release of the drug from the antibody. However, these carbohydrate-based conjugates suffer from various drawbacks. First, it is necessary to use periodate to generate aldehydes from the carbohydrate residues of the antibody. Antibodies contain cysteines, cystines, methionines, tryptophans, or tyrosines residues which are necessary for proper functioning of the antibody. However, these same amino acids can be sensitive to periodate oxidation, and if such oxidation takes place to an amino acid which either is part of the antigen binding site of the antibody or a structurally important region near the antigen binding site, its immunoaffinity can.be significantly diminished. A second drawback of using the carbohydrates for conjugation is the variability of the hydrazones and related structures that are generated from the naturally-occurring sugars and the hydrazide derivative. Not only are the hydrazones subject to different rates of hydrolysis due to differences in their local structure, but other structures, such as hydrated species, piperadines, etc. can also be generated. Any one conjugate may contain structures that are either too stable or too labile for optimum activity.

[0027] Limited examples of how to combine some of the properties of the carbohydrate-based conjugates and the lysine-based conjugates have appeared using other less potent classes of anticancer agents. Cullinan in U.S. Pat. No. 5,006,652 and 5,094,849 teaches that certain bifunctional compounds containing both carboxylic acid and aldehyde or keto functionality can be used as spacers between the lysines of antibodies and hydrazide derivatives of the Vinca alkaloids, while Johnson in U.S. Pat. No. 5,028,697 and 5,144,012 teaches similar art for methotrexate analogs. Sinam et al. also disclose similar constructs in WO Pat. No. 90/03401. In none of these cases is it demonstrated that this method is useful for preparing conjugates of the methyltrisulfide antitumor antibiotics, especially the calicheamicins or esperamicins. The cited patents do not demonstrate that these constructs made with either the Vinca alkaloids, the methotrexate analogs, or other agents are superior in their biological profile to conjugates made using lysine-based or carbohydrate-based conjugates.

[0028] The present invention describes a series of conjugates prepared from the potent methyltrisulfide antitumor antibiotics made with an improved linker system that gives conjugates which in many cases are vastly superior biologically to conjugates of the same drugs made by other methods.

DETAILED DESCRIPTION OF THE INVENTION

[0029] The conjugates of this invention use linkers that can be added to a derivative of a drug, particularly hydrazides and related nucleophiles, prepared from the methyltrisulfide containing antitumor antibiotics. The linkers require a carbonyl group on one end for formation of a Schiff's base, particularly a hydrazone, and a carboxylic acid on the other end. The carboxylic acid can be activated and subsequently reacted with the lysines of an antibody or other targeting protein or with an amine, alcohol, or other appropriate nucleophile on other targeting agents which have been chosen for their ability to target undesired cell populations. These constructs, which for antibodies contain elements of both the lysine-based conjugates and the carbohydrate-based conjugates, not only overcome the disadvantages of previously disclosed constructs, but have the additional advantage that they can be fine-tuned by varying the structure of the linker to "design in" the optimum amount of hydrolytic stability/instability. This can result in maximum toxicity to the target cells with minimal toxicity to the non-target cells. The optimum hydrazone stability/instability is not necessarily the same for each drug and targeting agent combination.

[0030] The method of constructing the conjugates described in this patent produces conjugates of the methyltrisulfide antitumor antibiotics which are unexpectedly stable relative to the carbohydrate based conjugates without loss of activity. In some cases, the conjugates are 100 times more potent than the corresponding conjugates made by the carbohydrate-based method and, in addition, show reduced cytotoxicity against non-target cell lines. This results in conjugates with up to 10,000-fold selectivity between target and non-target cell lines.

[0031] The linkers required for the construction of these conjugates can be represented by the following formula:

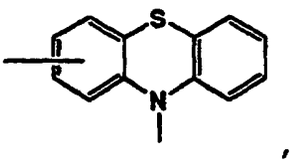
$$Z^3[CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=Z^2]_m$$

[0032] Alk^1 and Alk^2 are independently a bond or branched or unbranched (C_1-C_{10}) alkylene chain. Sp^1 is a bond, -S-, -O-, -CONH-,-NHCO-, -NR'-, $-N(CH_2CH_2)_2N-$, or -X-Ar'-Y-$(CH_2)_n$-Z wherein n is an integer from 0 to 5, X, Y, and Z are independently a bond, -NR'-, -S-, or -O-, and Ar' is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, COOR', CONHR', $O(CH_2)_nCO$-OR', $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ wherein n is as hereinbefore defined, with the proviso that when Alk^1 is a bond, Sp^1 is also a bond. R' is a branched or unbranched (C_1-C_5) chain optionally substituted by one or two groups of -OH, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, (C_1-C_3) dialkylamino, or (C_1-C_3) trialkylammonium - A^- where A^- is a pharmaceutically acceptable anion completing a salt. Sp^2 is a bond, -S-, or -O-, with the proviso that when Alk^2 is a bond, Sp^2 is also a bond. Z^3 is a hydroxyl group, and m is 1.

[0033] The groups Alk^1, Sp^1, Alk^2 and Sp^2 in combination, as well as the group Ar discussed below, allow for spacing of the carbonyl group from the carboxylic acid. Furthermore, Alk^1 and Sp^1 can influence the reactivity of the carboxyl group both during and after it has been activated. When Alk^2 and Sp^2 together are a bond, the Sp^1 group also influences the reactivity of the carbonyl group on the other end of the linker and the stability of the product formed from reactions at that carbonyl. The group R' can be used to influence the solubility and other physiochemical properties of these compounds. A preferred embodiment for Alk^1 is (C_2-C_5) alkylene, and for Sp^1 is an oxygen atom. A preferred embodiment for the groups Alk^2 and Sp^2 together is a bond.

[0034] With reference to the structure shown above, the group Z^2 is an oxygen atom. The group Z^1 is H, (C_1-C_5) alkyl, or phenyl optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ wherein n and R' are as hereinbefore defined. The group Z^1 has a pronounced effect on the reactivity of the carbonyl group and on the stability of the products formed from reactions at the carbonyl. When Z^1 is aryl and the product is, for example, a hydrazone, the hydrazone is relatively stable; when Z^1 is hydrogen, then an intermediate level of stability is obtained, and when Z^1 is (C_1-C_6) alkyl, relatively less stable hydrazones are formed. As stated earlier, stability is important to prevent premature release of the drug from the antibody, but some instability is needed to allow release of the drug once the conjugate has been internalized into target cells. A preferred embodiment for the Z^1 group is (C_1 to C_3).

[0035] The group Ar is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ wherein n and R' are as hereinbefore defined or a 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6-, or 2,7-naphthylidene or



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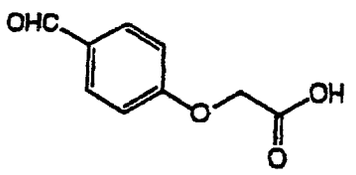
wherein Sp^1 a bond only connected to the nitrogen of the phenothiazine, each optionally substituted with one, two, three, or four groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, or COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ wherein n and R' are as hereinbefore defined;

[0036] The choice of Ar has a significant influence on the stability of the products derived from the carbonyl when Alk^2 and Sp^2 are together a bond. Both the relative position of Sp^1 and Sp^2 as well as the presence of additional substituents on Ar can be used to fine-tune the hydrolytic behavior of the product formed from the carbonyl. A preferred embodiment for Ar is 1,2-, 1,3-, or 1,4-phenylene, or 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6-, or 2,7-naphthylidene.

[0037] The structures of specific examples of linkers which are useful in the present invention are as follows:



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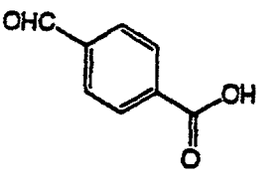

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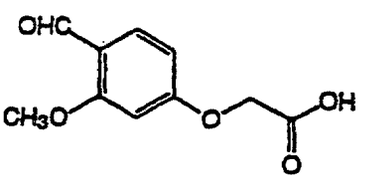

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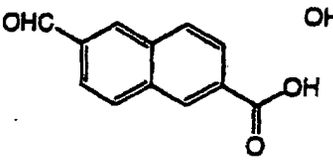

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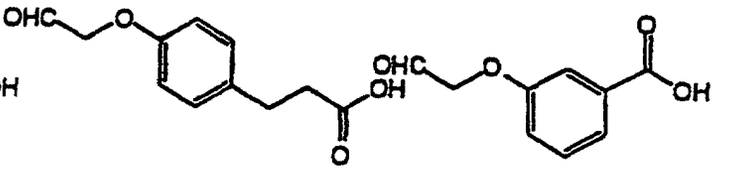

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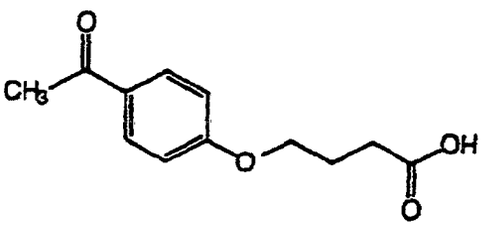

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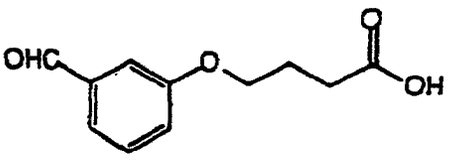

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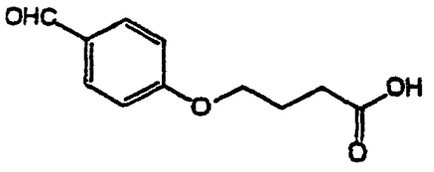

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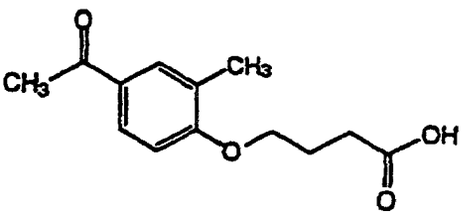

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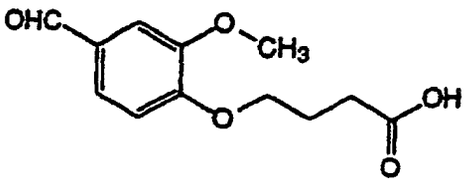

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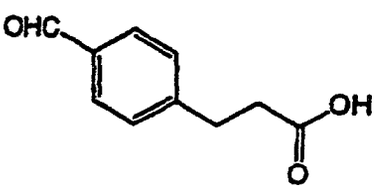

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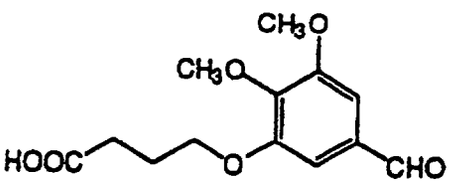

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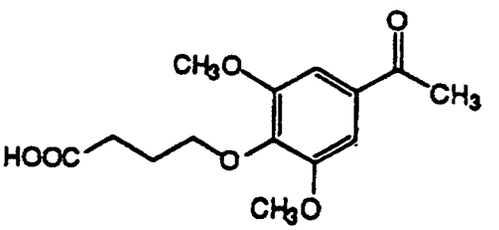

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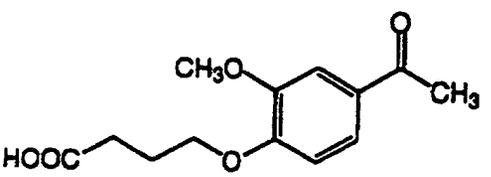

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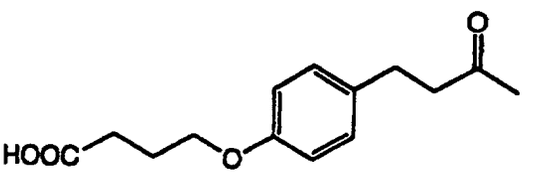

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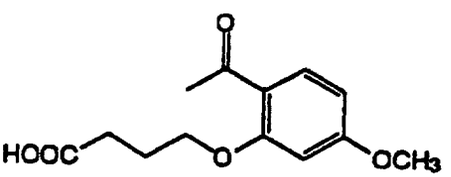

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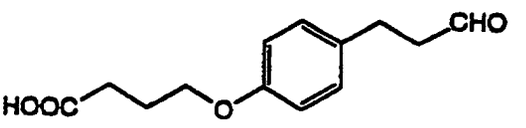

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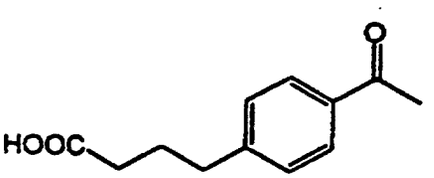

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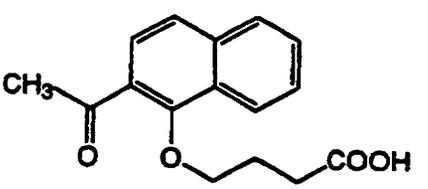

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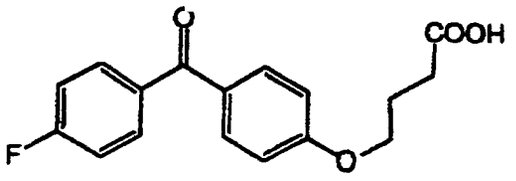

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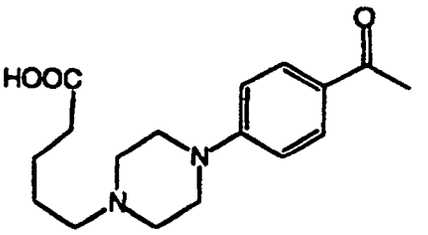

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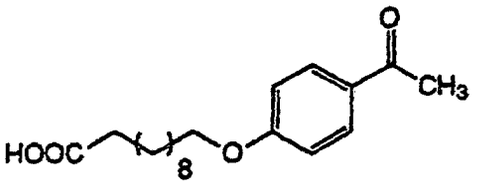

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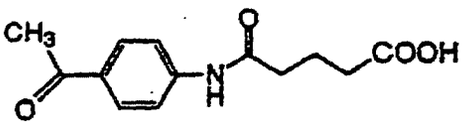

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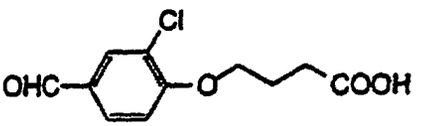

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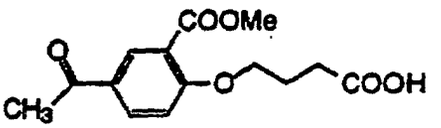

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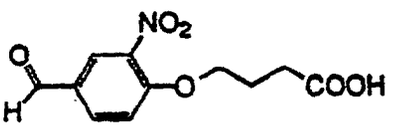

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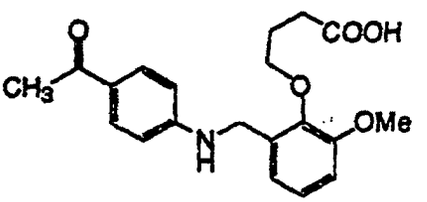

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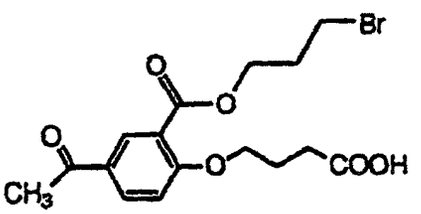

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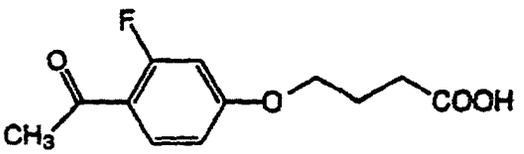

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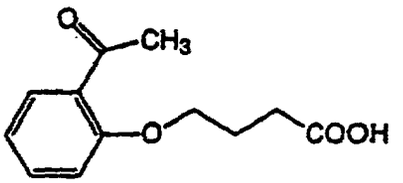

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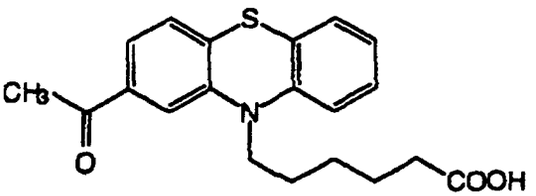

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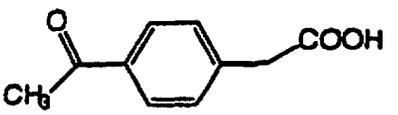

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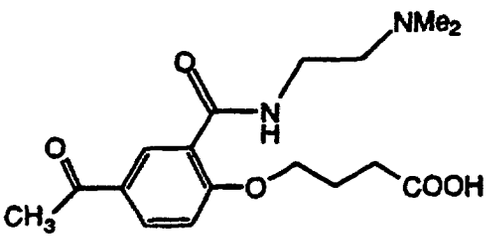

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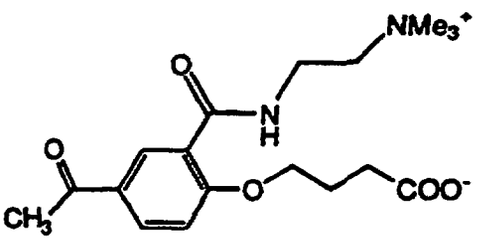

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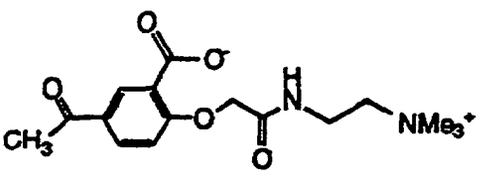

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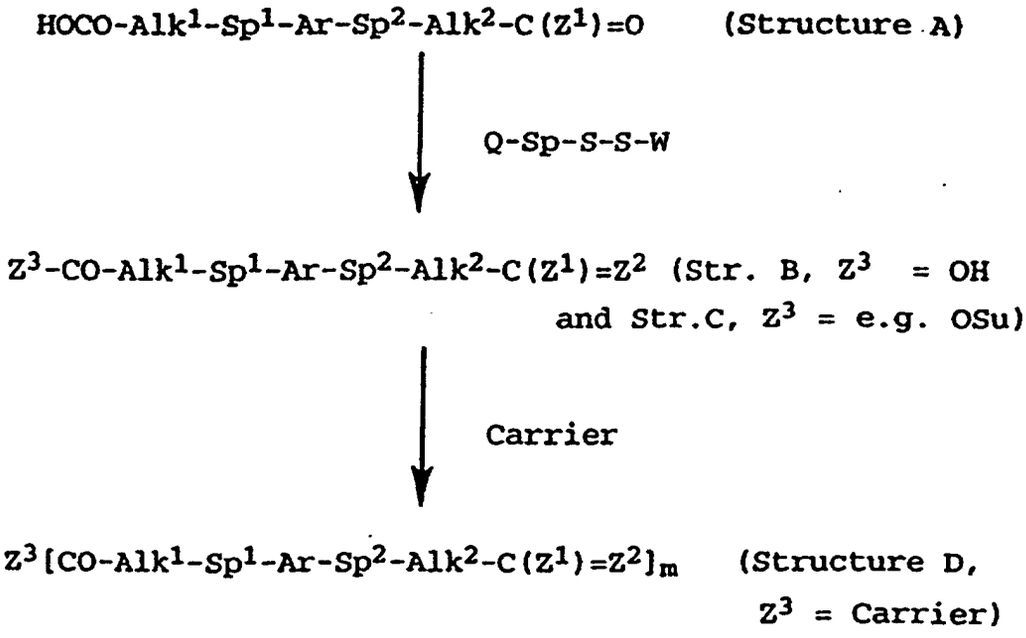
[0038] Only a few of the more simple of these linkers are commercially available, i.e., linkers 1, 2, 3, 19, 23, 24 and 33. Linker 20 is listed by the Chemical Abstract Services with registry number 5084-45-7. Many linkers which contain aryl ethers as a part of their structure, such as 7, 8, 10, 13, 14, 15, 16, 17, 20, 21, 25, 28, 30, and 31, can be made by alkylating a phenolic ketone with an electrophile, such as ethyl 4-bromobutyrate, using an appropriate base, such as potassium carbonate, in an appropriate solvent, such as N,N-dimethyl formamide, and then converting the ester into the required carboxylic acid by hydrolysis with, for example, sodium hydroxide or potassium carbonate in aqueous methanol. This strategy can also be used with linkers such as 5, 6, 9, 11, 18, or 27, where the carbonyl is carried through the initial steps of the preparation in a masked form, such as an olefin or an alcohol. The carbonyl can then be generated later, as described in the examples, by oxidation with ozone or pyridinium chlorochromate, resp. This procedure is especially valuable when a more reactive carbonyl is present in the final linker.

[0039] When necessary, the required carboxylic acid can be introduced in a masked form as in the preparation of linker 26. In this case the phenol is alkylated with 5-bromo-1-pentene and the acid is liberated from the olefin by reaction

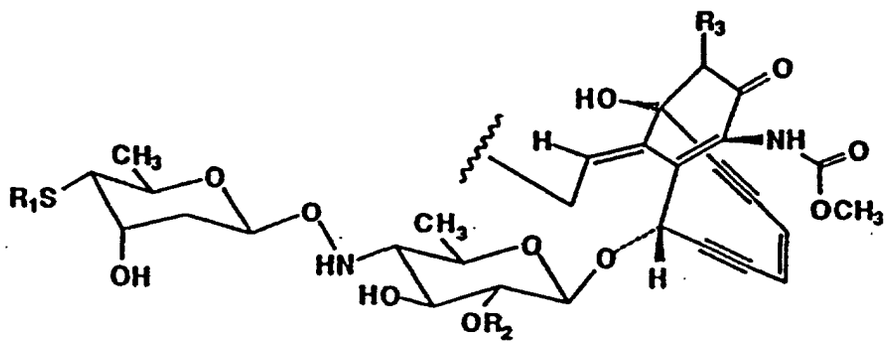
with ozone followed by pyridinium chlorochromate oxidation. Linkers such as 22 or 32 can be made by alkylating an appropriate secondary amine (a piperazine or phenothiazine derivative, resp.) with an appropriate electrophile and then exposing the required carboxylic acid in a later step, similar to the previously mentioned strategies. Linker 12 was made by reduction of the corresponding cinnamate with hydrogen. Although this reaction gave a relatively impure product, the crude mixture was useful for conversion to the required hydrazone because none of the by-products contained aldehyde groups. Structures with more elaborate substituents, such as linkers 33, 34, 35, or 36, can be made from simpler structures by, for example, reacting an ester with an appropriate nucleophile or by quaternizing an amine with an electrophile, such as methyl iodide.

[0040] The linkers defined above can be used to form conjugates as follows:

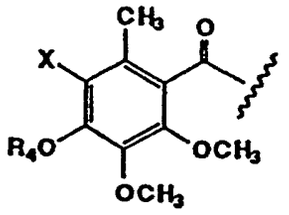


Scheme 1

With reference to Scheme 1 above, the linker of structure A, wherein Z^1, Alk^1, Sp^1, Ar, Sp^2, and Alk^2 are as hereinbefore defined, is condensed with a compound of structure Q-Sp-S-S-W which itself is derived from a methyltrithio antitumor antibiotic, and wherein W is

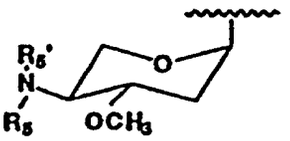


R_1 is



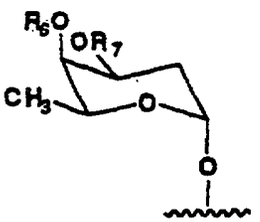
or CH_3;

R_2 is



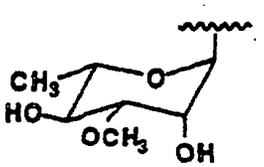
or H;

R_3 is



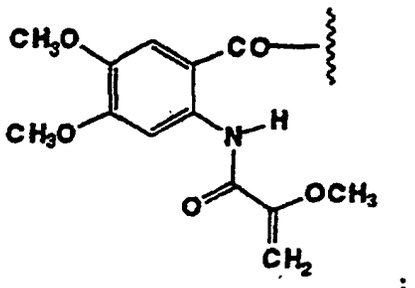
or H;

R_4 is



or H;

R_6 or R_7 is H or

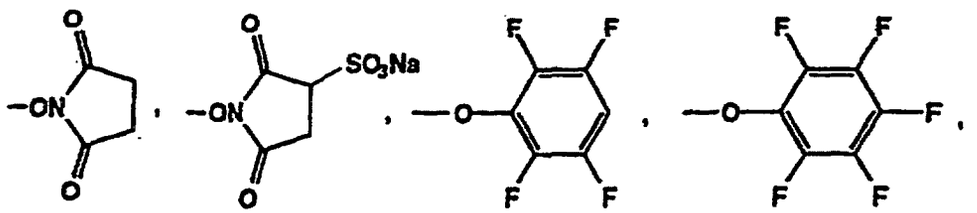


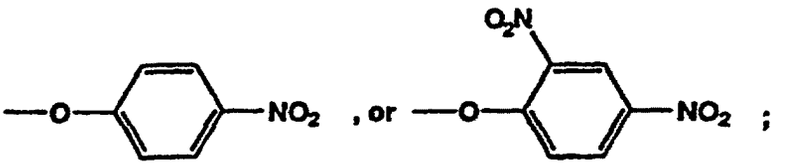
;

[0041] R_5 is $-CH_3$, $-C_2H_5$, or $-CH(CH_3)_2$; X is an iodine or bromine atom; R_5' is a hydrogen or the group RCO, wherein R is hydrogen, branched or unbranched (C_1-C_{10}) alkyl or (C_1-C_{10}) alkylene group, a (C_6-C_{11}) aryl group, a (C_6-C_{11}) aryl-alkyl (C_1-C_5) group, or a heteroaryl or heteroaryl-alkyl (C_1-C_5) group wherein heteroaryl is defined as 2- or 3-furyl, 2- or 3-thienyl, 2- or 3-(N-methylpyrrolyl), 2-, 3-, or 4-pyridinyl, 2-, 4-, or 5-(N-methylimidazolyl), 2-, 4-, or 5-oxazolyl, 2-, 3-, 5-, or 6-pyrimidinyl, 2-, 3-, 4-, 5-, 6-, 7-, or 8-quinolyl, or 1-, 3-, 4-, 5-, 6-, 7-, or 8-isoquinolyl, all aryl and heteroaryl optionally substituted by one or more hydroxy, amino, carboxy, halo, nitro, lower (C_1-C_3) alkoxy, or lower (C_1-C_5) thioalkoxy groups; Sp is a straight or branched-chain divalent or trivalent (C_1-C_{18}) radical, divalent or trivalent aryl or heteroaryl radical, divalent or trivalent (C_3-C_{18}) cycloalkyl or heterocycloalkyl radical, divalent or trivalent aryl- or heteroaryl-alkyl (C_1-C_{18}) radical, divalent or trivalent cycloalkyl- or heterocycloalkyl-alkyl (C_1-C_{18}) radical or divalent or trivalent (C_2-C_{18}) unsaturated alkyl radical, wherein heteroaryl is furyl, thienyl, N-methylpyrrolyl, pyridinyl, N-methylimidazolyl, oxazolyl, pyrimidinyl, quinolyl, isoquinolyl, N-methylcarbazoyl, aminocoumarinyl, or phenazinyl and wherein if Sp is a trivalent radical, it can be additionally substituted by lower (C_1-C_5) dialkylamino, lower (C_1-C_5) alkoxy, hydroxy, or lower (C_1-C_5) alkylthio groups; and Q is H_2NHNCO-, H_2NHNCS-, $H_2NHNCONH$-, $H_2NHNCSNH$-, or H_2NO-, to produce a compound of structure B, wherein Z^1, Alk^1, Sp^1, Ar, Sp^2, and Alk^2 are as hereinbefore defined, Z^2 is Q-Sp-S-S-W, wherein Sp and W are as herein above defined, Q is =NHNCO-, =NHNCS-, =NHNCONH-, =NHNCSNH-, or =NO-, and Z^3 is -OH.

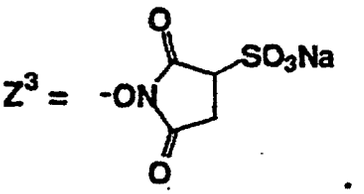
[0042] The condensation can be run in most compatible organic solvents, but is particularly efficient in alcoholic solvents such as methanol or ethanol. This condensation reaction is acid catalyzed. The carboxylic acid in the linkers themselves is sufficient in many cases to catalyze this reaction, but adding a compatible acid catalyst, such as about 5% acetic acid, helps improve the rate of reaction in many cases. The temperature of this reaction can be from about ambient temperature to the reflux temperature of the solvent. The products are isolated in pure form by removing the volatile solvents and purifying the mixture by chromatography on a suitable medium such as BioSil A™, a modified silica gel available from Bio-Rad. It should be understood that the products of structure B, as well as the products from the further transformation of these compounds, exist as easily-interconverted *syn* and *anti* isomers at the locus defined as Q, and that these products can exist in different hydrated forms, depending on the exact conditions of solvent and the pH at which these compounds are examined. Such differing physical forms are also included within the scope of this patent.

[0043] The carboxylic acid of structure B (Z^3 = -OH) is next converted to an activated ester in preparation for conjugation of these intermediates with carrier molecules. Such transformations convert Z^3 (structure B) to halogen, $-N_3$,





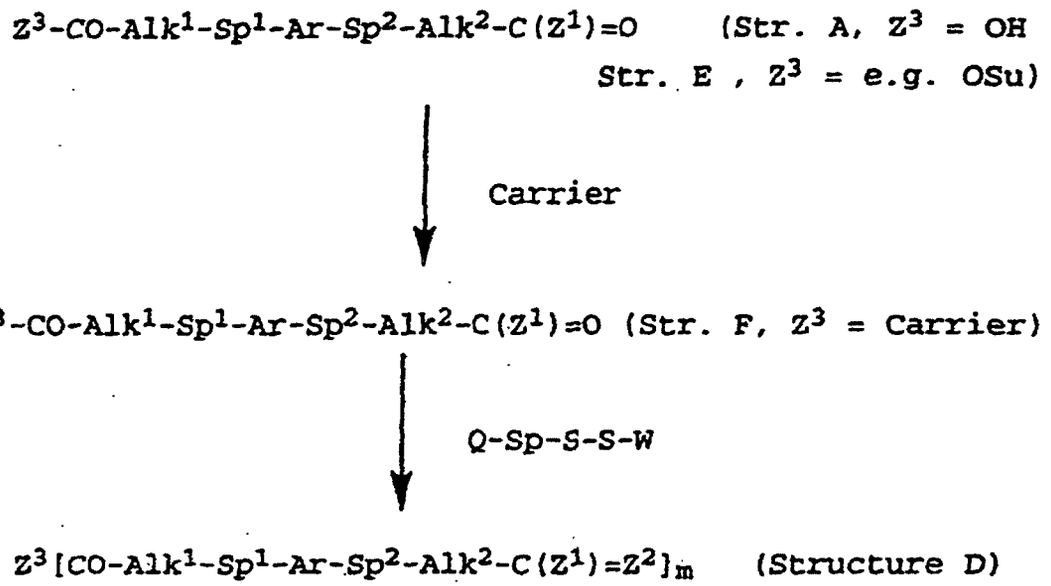
[0044] For example, reaction of the carboxyl form of structure B (Z^3 = -OH) with a coupling agent, such as 1,3-dicyclohexylcarbodiimide or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, and N-hydroxysuccinimide or other comparable carboxyl-activating group in an inert solvent, such as N,N-dimethylformamide, tetrahydrofuran, or acetonitrile, leads to the formation of an activated ester, such as the N-hydroxysuccinimide ester described herein. These active esters can be isolated in pure form by removal of the volatile solvents and chromatography on an appropriate medium, such as BioSil A™. Alternately, the coupling reaction can be quenched with a polymeric carboxylic acid, filtered, and stripped of organic solvents, and the crude product can be used in the following step without further purification. This is especially useful if the active ester is difficult to handle, such as when



[0045] The final step in the construction of the conjugates of this patent involves the reaction of an activated ester (structure C) with a targeting molecule, as shown in Scheme 1. This produces a compound of structure D, wherein Z^1, Z^2, Alk^1, Sp^1, Ar, Sp^2, and Alk^2 are as hereinbefore defined, m is 0.1 to 15, and Z^3 is a protein such as a growth factor or a mono- or polyclonal antibody, their antigen-recognizing fragments, or their chemically or genetically manipulated counterparts or a steroid, wherein a covalent bond to a protein is an amide formed from reaction with "m" lysine side chains and the covalent bond to a steroid is an amide or an ester.

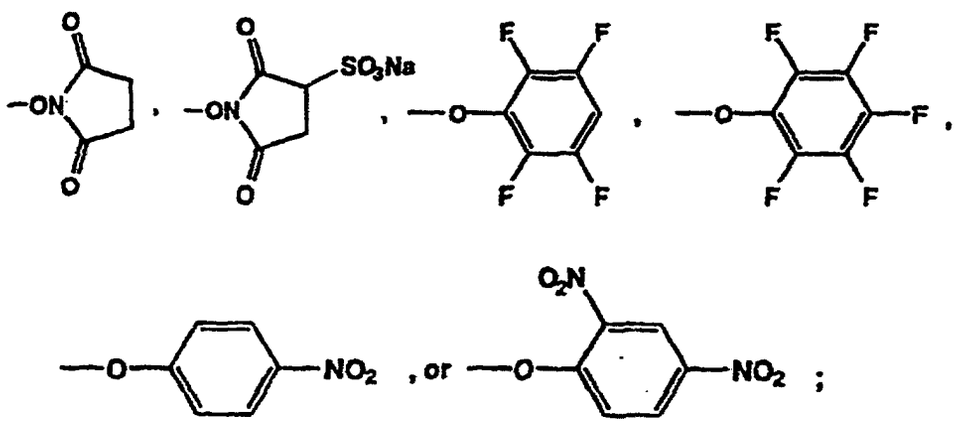
[0046] This conjugation reaction can be carried out in various appropriate buffers, such as borate, phosphate, or HEPES at slightly basic pH (pH ~7.4 to 8.5). The final construct can then be purified by appropriate methods, such as gel-exclusion chromatography, to remove unattached drug and aggregates to yield monomeric conjugates. This sequence of steps constitutes Method A as described in greater detail in the Examples section of this patent.

[0047] Alternative methods for constructing the conjugates of Scheme 1 are also contemplated as shown in Scheme 2.



Scheme 2

[0048] For example, the linker (structure A as defined above) can be converted to an active ester and reacted with the targeting molecule prior to the reaction with the drug. Such manipulations convert structure A into structure E, wherein Z^1, Alk^1, Sp^1, Ar, Sp^2, and Alk^2 are as hereinbefore defined, Z^2 is an oxygen atom, and Z^3 is halogen, $-N_3$,



[0049] The activated ester is then reacted with the carrier to produce structure F, wherein Z^1, Alk^1, Sp^1, Ar, Sp^2, and Alk^2 are as hereinbefore defined, Z^2 is an oxygen atom, m is about 1 to about 20, and Z^3 is a protein selected from mono- and polyclonal antibodies, their antigen-recognizing fragments, and their chemically or genetically manipulated counterparts and growth factors and their chemically or genetically manipulated counterparts, wherein a covalent bond to the protein is an amide formed from reaction with "m" lysine side chains, or a steroid, wherein the covalent bond to the steroid is an amide or an ester;.

[0050] Once the targeting molecule has been modified with the linker, it can be reacted with a compound of structure Q-Sp-S-S-W, which itself is derived from a methyltrithio antitumor antibiotic, and wherein W and Sp are as hereinbefore defined, and Q is H_2NHNCO-, H_2NHNCS-, $H_2NHNCONH$-, $H_2NHNCSNH$-, or H_2NO- to produce a compound of Structure D (*vida supra*).

[0051] This sequence of steps in Scheme 2 constitutes Method B in the Examples section of this patent. Similar antibody-carbonyl constructs are covered in U.S. Pat. No. 5,144,012 mentioned above. Most of the linkers exemplified

herein are new and offer the advantage that a much broader range of structural types and hence a broader range of stabilities is demonstrated. As a specific example, the acetophenone linkers, which are new to this patent, produced conjugates with better hydrolytic release properties of drug and which are more potent when used with the examples of the antibodies shown here. Specifically, the two conjugates prepared from h-P67.6 using 4-formylbenzenepropanoic acid or 4-acetylbenzenebutanoic acid condensed with calicheamicin N-acetyl gamma hydrazide (the two conjugates only differ by having Z^1 = -H and Z^1 = $-CH_3$, respectively, in structure 3 of Figure 1) gave *in vitro* IC_{50}'s of 1.0 and 0.012 ng/mL, and specificity indices of 950 and 26,000, resp. Although the acetophenone based linkers are seen to be superior in this case, it is not necessarily easy to predict which linker will be superior for any given targeting agent-drug construct.

BIOLOGICAL CHARACTERIZATION

[0052] Assessment of the biological properties of the conjugates included measuring their ability to recognize the antigen on target cell lines, relative to the unmodified antibody, and determining their selectivity and cytotoxic potentials, using the following methods:

IMMUNOAFFINITY ASSAYS

[0053] Relative immunoaffinities of conjugates are determined in a competitive binding assay in which varying concentrations of test conjugate are allowed to compete with a fixed amount of the same antibody labeled with ^{125}I-Bolton Hunter reagent for binding to a fixed number of cells. For m- or h-P67.6, HEL 92.1.7 human erythroleukemia cells [ATCC (American Type Culture Collection) TIB 180] are used at a concentration of 10^7 cells/mL; for CT-M-01, cell line A2780DDP (E.M. Newman, et al., "Biochem. Pharmacol." **37**, 443 (1988)) is used; and for m- or h-A33, cell line COLO 205 (ATCC CCL 222) is used. The concentration of test conjugate required to obtain 50% inhibition of binding of the labeled antibody to target cells is compared with the concentration of a reference preparation of native antibody required for 50% inhibition.

[0054] samples for assay are adjusted to ~300 μg protein/mL in medium and six serial four-fold dilutions of each are prepared in medium (RPMI-1640 containing 5% heat-inactivated fetal calf serum), for a total of seven concentrations of each sample. The reference antibody is diluted in the same way. An aliquot of 0.05 mL of each dilution is transferred to a 12 X 75 mm plastic tube, and 0.05 mL of labeled reference antibody at 4 μg/mL is added. The tubes are mixed and chilled at 4°C. Then 0.1 mL of chilled cell suspension is added to each tube. All tubes are mixed again, and incubated for 1 hr at 4°C.

[0055] Controls to determine maximal binding and non-specific binding are included in each assay. Maximal binding is determined by mixing 0.05 mL of medium, 0.05 mL of ^{125}I-antibody, and 0.1 mL of cells; non-specific binding is determined by mixing 0.05 mL of 500 μg/mL of native antibody, 0.05 mL of iodinated antibody, and 0.1 mL of cells.

[0056] At the end of the incubation, cells are washed twice, by centrifugation and resuspension, with 3 mL of cold PBS each time. The cells are resuspended in 0.5 mL of PBS, transferred to clean tubes, and radioactivity is determined in a gamma-counter.

[0057] The percent inhibition of binding is calculated by the following equation:

$$\%I = \{[(cpm_{maxbinding} - cpm_{non\text{-}specific}) - (cpm_{sample} - cpm_{non\text{-}specific})] + - (cpm_{max\ binding} - cpm_{non\text{-}specific})\} X\ 100$$

The percent inhibition values are plotted against sample concentrations, and from the resulting curves the sample concentration that gives 50% inhibition of binding (IC_{50}) is interpolated. The relative immunoaffinity of each tested conjugate is then determined as follows:

$$\text{Relative Immunoaffinity} = \frac{IC_{50}(\text{reference})}{IC_{50}(\text{sample})}$$

IN VITRO CYTOTOXICITY ASSAY

[0058] Cytotoxic activities are determined in an *in vitro* pulse assay in which varying concentrations of test conjugate

are incubated with antigen-positive and antigen-negative cells for 1 hr, then cultured for three days. Viability is assessed by [^{3}H]thymidine incorporation during the final 24 hr of culture. As a measure of potency, the concentration of test conjugate required to inhibit [^{3}H]thymidine incorporation by 50% (IC_{50}) is. determined from the titration curve. The specificity is determined by comparing IC_{50} values on antigen-positive and antigen-negative cells for P67.6, A33, and m-CT-M-01 or by use of a conjugate of the same drug with the non-targeting antibody P67.6 for h-CT-M-01 conjugates or MOPC-21 for anti-Tac conjugates. MOPC-21 (F. Melchers, "Biochem. J." **119,** 765 (1970)) is an antibody which does not recognize any normally occurring, physiologically pertinent antigen.

[0059] For P67.6, antigen-positive HL-60 human promyelocytic leukemia cells (ATCC CCL 240) and antigen-negative Raji human Burkitt lymphoma cells (ATCC CCL 86) are used; for A33, antigen-positive COLO 205 cells and antigen-negative Raji cells are used; and for h-CT-M-01, ZR-75-1 cells (ATCC CRL1500) are used. For m-CT-M-01 antigen-positive A2780DDP cells and antigen-negative Raji cells are used, and for h-CT-M-01, ZR-75-1 cells (ATCC CRL1500) are used. Cells are collected by centrifugation, counted, and resuspended in fresh medium. (RPMI-1640 + 5% heat-inactivated fetal calf serum + antibiotics) at a cell concentration of ~10^6/mL.

[0060] Samples for assay are readjusted to ~1 μg/mL of drug equivalents in medium and five serial ten-fold dilutions of each are prepared in medium, for a total of six concentrations of each sample. In addition, a medium control is included with each sample set, as well as calicheamicin N-acetyl gamma as a drug control. An aliquot of 0.1 mL of cell suspension is added to 17 X 100 mm plastic tubes containing 0.1 mL of sample; a separate series of tubes is prepared for each cell line. The tubes are loosely capped and incubated for 1 hr at 37°C in a humidified atmosphere of 5% CO_2 in air. At the end of the incubation, cells are washed twice by centrifugation and resuspended with 8 mL of medium each time. Cell pellets are resuspended in 1 mL of medium and plated in triplicate in 96-well microtiter plates at 0.2 mL/well. The plates are incubated for 2 days at 37°C as above. Then 0.1 mL of medium is removed from each well and replaced with 0.1 mL of fresh medium containing 0.1 μCi of [^{3}H]thymidine. Plates are returned to the incubator for one more day. Plates are frozen and thawed, and cells are harvested on glass fiber filter mats. The amount of [^{3}H] thymidine incorporated is determined by liquid scintillation counting.

[0061] The measured cpm of the triplicate cultures of each sample dilution are averaged and the percent inhibition of [^{3}H]thymidine incorporation is calculated by the following equation, where the values for no inhibition and maximal inhibition come from the medium controls, and the highest concentration of calicheamicin N-acetyl gamma, respectively:

$$\%I = \{[(cpm_{\text{no inhibition}} - cpm_{\text{max inhibition}}) - (cpm_{\text{sample}} - cpm_{\text{max inhibition}})] + (cpm_{\text{no inhibition}} - cpm_{\text{max inhibition}})\} \times 100$$

[0062] The percent inhibition values are plotted against sample concentrations, and from the resulting curves the sample concentration that gives 50% inhibition of [^{3}H]thymidine incorporation (IC_{50}) is interpolated. For P67.6, A33, and m-CT-M-01 conjugates, the specificity of a particular conjugate for antigen-positive cells is calculated by taking the ratio of the IC_{50} against non-target cells to the IC_{50} against target cells. The same ratio is calculated for the free drug. Then, to correct for inherent differences in the sensitivities of the two cell lines to the drug, the Specificity Index for each sample is calculated as follows:

$$\text{Specificity Index} = [IC_{50\ (\text{sample on antigen neg})} + IC_{50\ (\text{sample on antigen pos})}] + [IC_{50\ (\text{drug on antigen neg})} + IC_{50\ (\text{drug on antigen pos})}]$$

[0063] For conjugates of Anti-Tac or h-CT-M-01, the Specificity Index is calculated as the ratio of IC_{50}'s for the non-targeting conjugate and the targeting conjugate as follows:

$$\text{Specificity Index} = \frac{IC_{50\ (\text{non-targeting conjugate})}}{IC_{50\ (\text{targeting conjugate})}}$$

***IN VIVO* ANTITUMOR ASSAY**

[0064] Human tumors (either ~10^7-10^8 cells or 5 to 8-2 mm^3 fragments of solid tumors) are implanted subcutaneously into athymic mice (nude mice) and test samples are inoculated intraperitoneally (ip) at several dose levels on a q 4 day x 3 schedule, starting 2-3 days after tumor implantation with 5 mice per test group and 10 in the saline control group. Tumor mass is estimated by measuring the tumor length and width once weekly up to 42 days post tumor implantation with a Fowler ultra CAL II electronic caliper and using the formula: mg tumor = {Length(mm) x Width(mm)} /2. Tumor growth inhibition is calculated as the ratio of the mean tumor mass of treated animals compared with untreated controls and is expressed as "% T/C". (0% T/C implies no detectable tumor. All control animals, routinely develop easily measurable tumor.)

***EX VIVO* INHIBITION OF COLONY FORMATION**

[0065] For P67.6 conjugates, human leukemic bone marrow cells which are CD-33 positive are plated in the presence of 2 ng/mL drug equivalents. The number of colonies which form are counted and reported as the percent versus a control which consists of a h-CT-M-01 conjugate which does not recognize the CD-33 antigen. All the data reported were generated with bone marrow from one patient whose leukemic cells had good antigen expression and good response to this general type of treatment.

[0066] For anti-Tac, peripheral blood from CML patients was tested. Progenitor cells for cells of the various hematopoietic lineages can be detected by culturing bone marrow cells and blood cells in a semisolid matrix such as methylcellulose and observing the formation of colonies containing mature differentiated cells. There are progenitor cells that proliferate to form colonies of granulocytes or macrophages, or both, called colony-forming units for granulocytes-macrophages (CFU-GM). Some CFU-GM form colonies within seven days (D7 CFU-GM); some require fourteen days for colony formation (D14 CFU-GM) [N. Jacobsen, *et al.*, "Blood" 52: 221, (1978), and Ferrero D et al. "Proc. Natl. Acad. Sci. USA" 80: 4114, (1983)]. Inhibition of the growth of D14 CFU-GM on blood cells treated with anti-Tac was compared to those treated with non-targeting MOPC 21 conjugates. The number of D14 CFU-GM colonies are plotted against sample concentrations, and from the resulting curves the sample concentration that gives 50% inhibition of D14 CFU-GM colony growth is interpolated. Specificity was measured by the ratio of the IC_{50} of the non-targeting conjugate versus the IC_{50} of the targeting conjugate. Normal blood does not produce CFU-GM colonies and normal bone marrow D14 CFU-GM colonies are not inhibited by anti-Tac conjugates.

[0067] The invention is further described with the following non-limiting preparations and examples. (Preparations describe the syntheses of compounds useful in this invention but for which there is known prior art. Examples describe the syntheses of compounds which are useful and new to this invention.)

SYNTHESIS OF STRUCTURES A (Scheme 1 and Scheme 2)

EXAMPLE 1, COMPOUND **5**

4-(2-Oxoethoxy)benzenepropanoic acid

[0068] 4-Hydroxybenzenepropanoic acid (500 mg, 3.01 mmol) is allowed to react with 910 mg (7.52 mmol) of allyl bromide by the same procedure described in Example 2 to give, 610 mg (82%) of 2-propenyl-4-(2-propenyloxy)-benzenepropanoic ester as a colorless oil. The product is utilized in the next reaction without further purification. The ^1H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (neat) 3450, 1740, 1650, 1610, 1510 cm^{-1}; MS (CI low res) m/e 247 (M+H), 229, 215, 187, 175; Analysis calc'd for $C_{15}H_{18}O_3$: C, 73.15; H, 7.37; found: C, 73.09; H, 6.74.

[0069] 2-Propenyl-4-(2-propenyloxy) benzenepropanoic ester (271 mg, 1.1 mmol) is treated with 0.14 mL (1.38 mmol) of 10 M sodium hydroxide solution according to the same procedure described for Example 2 to give 196 mg (86%) of 4-(2-propenyloxy)benzenepropanoic acid as a white powder. The product is utilized in the next reaction without further purification: m.p. 88-89°; the ^1H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (KBr) 3200, 1720, 1700, 1610 cm^{-1}; MS (CI low res) m/e 207 (M+H), 189, 175, 147; Analysis calc'd for $C_{12}H_{14}O_3$: C, 69.89; H, 6.84; found: C, 69.87; H, 6.68.

[0070] 4-(2-Propenyloxy)benzenepropanoic acid (120 mg, 0.58 mmol) is treated with ozone by the procedure described in Example 2 to give 100 mg (82%) of 4-(2-oxoethoxy)benzenepropanoic acid as a white powder: m.p. 95-100°; the ^1H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (KBr) 3400, 1740, 1720, 1610 cm^{-1}; MS (CI low res) m/e 207, 191, 179, 165, 149.

EXAMPLE 2, COMPOUND **6**

3-(2-Oxoethoxy)benzoic acid

[0071] A mixture of 1.0 g (7.24 mmol) of 3-hydroxy-benzoic acid, 3.0 g (25.3 mmol) of allyl bromide, and 5 g (36.2 mmol) of potassium carbonate in 4 mL of N,N-dimethylformamide is stirred at room temperature for 12 hr. The mixture is diluted with 20 mL of ether and washed five times with 20 mL of water. The organic layer is then washed successively with 20 mL of saturated sodium bicarbonate solution and 20 mL of saturated sodium chloride solution. The organic layer is separated and dried over magnesium sulfate. The mixture is filtered and the organic solution is concentrated *in vacuo* to give 1.4 g (88%) of 3-(2-propenyloxy)benzoic acid, 2-propenyl ester as a clear colorless oil. The product is utilized in the next reaction without further purification. The ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (KBr) 1720, 1650, 1600 cm^{-1}; MS (CI low res) m/e 219 (M+H), 203, 175, 161; Analysis calc'd for $C_{13}H_{14}O_3$: C, 71.54; H, 6.47; found: C, 70.31; H, 5.97.

[0072] A solution of 917 mg (4.2 mmol) of 3-(2-propenyloxy)benzoic acid, 2-propenyl ester in 9 mL of methanol/water (3:2) at room temperature is treated with 0.53 mL (5.25 mmol) of 10 M sodium hydroxide solution. The solution is allowed to stir for one hr then acidified with 5 mL of 10% sodium bisulfate solution and extracted with 25 mL of ethyl acetate. The organic layer is washed with saturated sodium chloride solution, dried over magnesium sulfate, and concentrated in *vacuo* to give 732 mg (97%) of 3-(2-propenyloxy)benzoic acid as a white powder. The product is utilized in the next reaction without further purification; m.p. 78-79°; the ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (KBr) 3000, 1690, 1620, 1590 cm^{-1}; MS (CI low res) m/e 179 (M+H), 161, 135; Analysis calc'd for $C_{10}H_{10}O_3$: C, 67.41; H, 5.66; found: C, 67.37; H, 5.59.

[0073] A solution of 300 mg (1.68 mmol) of 3-(2-propenyloxy)benzoic acid in 5 mL of methylene chloride is cooled to -78° C. Ozone is introduced by bubbling the gas into the solution through a glass tube until a blue color persists. The solution is then purged with a stream of argon and 1 mL of methyl sulfide is added. The solution is diluted with 20 mL of ether and washed with water. The organic layer is separated and allowed to stand over magnesium sulfate then concentrated *in vacuo* to give 283 mg (93%) of 3-(2-oxoethoxy)benzoic acid as a colorless oil. The product is utilized in the next reaction without further purification: m.p. 120-130°; the ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (KBr) 3400, 3000, 1680, 1590 cm^{-1}; MS (CI low res) m/e 181 (M+H), 163, 139, 119.

PREPARATION 3, COMPOUND **7**

4-(4-Acetylphenoxy)butanoic acid

[0074] A solution of 0.90 g (6.61 mmol) of 4'-hydroxyacetophenone, and 1.93 g (9.92 mmol) of ethyl 4-bromobutyrate in 1.80 mL of N,N-dimethylformamide is stirred for 48 hr, under dry conditions with 2.74 g (19.8 mmol) of potassium carbonate and 0.110 g (0.66 mmol) of potassium iodide. The reaction mixture is then evaporated under vacuum, and the residue partitioned between ether and water. The organic phase is separated, washed thrice with water, dried with magnesium sulfate, filtered, and evaporated under vacuum to give a brown solid. This is recrystallized from a warm ether-hexane mixture. The beige crystals are air dried, leaving 0.84 g (51%) of 4-(4-acetylphenoxy)-butanoic acid, ethyl ester: m.p. 59-61° C; IR $^-$(KBr) 1740, 1670 cm^{-1}; ^{1}H-NMR ($CDCl_3$) is consistent with the desired product; MS (FAB) m/e 251.1285 Δ = -0.2 mmμ (M^+ + H). Anal. calc'd. for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25; O, 25.57. Found: C, 67.16; H, 7.16; O, 25.68.

[0075] A sample of 0.25 g (1.00 mmol) of 4-(4-acetylphenoxy)butanoic acid, ethyl ester (example 1) is dissolved in 15 mL of methanol/water (3:2), with stirring. Then, 0.21 g (1.50 mmol) of potassium carbonate is added and the reaction is stirred for 18 hr under an argon atmosphere. Next, the reaction mixture is evaporated under vacuum and the residue dissolved in 20 mL of a 0.1 N solution of sodium hydroxide. This basic solution is washed with ether, the aqueous phase acidified by addition of sodium bisulfate, and the resulting mixture extracted with ethyl acetate. This solution is then dried with magnesium sulfate, filtered and evaporated, leaving an off-white solid. This is crystallized from ethyl acetate with the addition of an equal volume of ether. This provides 0.18 g (80%) of 4-(4-acetylphenoxy)butanoic acid as light beige crystals: m.p. 148-50° C; IR (KBr) 1730, 1650 cm^{-1}; ^{1}H-NMR ($CDCl_3$) is consistent with the desired product; MS (FAB) m/e 223.0974 Δ = -0.4 mmμ (M^+ + H). Anal. calc'd. for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35; O, 28.80. Found: C, 64.61; H, 6.36; O, 29.03.

PREPARATION 4, COMPOUND 8

4-(3-Formylphenoxy)butanoic acid

[0076] 3-Hydroxybenzaldehyde (900 mg, 7.37 mmol) is treated with 2.16 g (11.05 mmol) of ethyl 4-bromobutyrate,

3.06 g (22.11 mmol) of potassium carbonate, and a catalytic amount (110 mg 0.74 mmol) of sodium iodide under the same conditions as in Preparation 3 to give a yellow oil. Purification by flash chromatography using hexane/ethyl acetate (10:1) gives 1.61 g of 4-(3-formylphenoxy)butanoic acid, ethyl ester as a light yellow oil. The ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (neat) 1730, 1700, 1600, 1580 cm^{-1}; MS (CI low res) m/e 237 (M+H), 191, 115.

[0077] A solution of 385 mg (1.63 mmol) of 4-(3-formylphenoxy)butanoic acid, ethyl ester and 850 mg (6.15 mmol) of potassium carbonate is stirred in 6 mL of methanol/water (3:2) at room temperature for 8 hr. The solution is then concentrated *in vacuo.* The residue is dissolved in 10 mL of 0.1N sodium hydroxide solution and washed with 20 mL of ether. The aqueous layer is separated and acidified with sodium bisulfate and extracted with ethyl acetate. The organic layer is washed with saturated sodium chloride solution, then dried over magnesium sulfate. The mixture is then filtered and concentrated *in vacuo* to give 315 mg of 4-(3-formylphenoxy)butanoic acid as a white solid. The product is utilized in the next reaction without further purification. m.p. 62-63°; the ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (KBr) 3400, 3000, 1700, 1690, 1590 cm-^{1}; MS (CI low res) m/e 209 (M+H), 191, 123.

PREPARATION 5, COMPOUND **9**

4-(4-Formylphenoxy)butanoic acid

[0078] 4-Hydroxybenzyl alcohol (1 g, 8.06 mmol) is treated with 1.73 g (8.86 mmol) of ethyl 4-bromobutyrate, 3.34 g (24.2 mmol) of potassium carbonate and a catalytic amount (120 mg 0.81 mmol) of sodium iodide under the same conditions as described in Preparation 3 to give 1.73 g of 4-[4-(hydroxymethyl)phenoxy]butanoic acid, ethyl ester as a light brown oil (90%). The product is utilized in the next reaction without further purification. The ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (neat) 3400, 1730, 1610, 1580, 1510 cm^{-1}; MS (CI) m/e 238, 221, 115.

[0079] A mixture of 230 mg (0.97 mmol) of 4-[4-(hydroxymethyl)phenoxy]butanoic acid, ethyl ester, 624.2 mg (2.9 mmol) of pyridinium chlorochromate, and a catalytic amount of 4 A molecular sieve is stirred in 2 mL of methylene chloride at room temperature for 3 hr. The mixture is diluted with 20 mL of ether, filtered and concentrated *in vacuo* to give 175 mg (76%) of a light yellow oil. The oil (150 mg, 0.63 mmol) is dissolved in 2.3 mL of methanol/water (3:2) and treated with 307 mg (2.22 mmol) of potassium carbonate according to the procedure described for Example 4 to give 100 mg (75%) of 4-(4-formylphenoxy)butanoic acid as a white powder. The product is used without further purification. The ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (KBr) 3000, 1740, 1660 cm^{-1}; MS (CI (low res)) m/e 209 (M+H), 191, 123.

PREPARATION 6, COMPOUND **10**

4-(4-Acetyl -2-methylphenoxy)butanoic acid

[0080] Utilizing the procedure of Preparation 3, 2.00 g (13.32 mmol) of 4-hydroxy-3-methylacetophenone is alkylated with ethyl 4-bromobutyrate. This produces 3.45 g (98%) of 4-(4-acetyl-2-methylphenoxy)butanoic acid, ethyl ester as a golden oil, after drying at 75° C, under vacuum: IR (neat) 1740, 1675 cm^{-1}; ^{1}H-NMR ($CDCl_3$) is consistent with the desired product; MS (FAB) m/e 265 (M^+ + H). Anal. calc'd. for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63; O, 24.21. Found: C, 67.92; H, 7.44; O, 24.64.

[0081] Following the method of Preparation 3, 2.50 g (9.46 mmol) of 4-(4-acetyl-2-methylphenoxy)butanoic acid, ethyl ester is saponified to give the desired compound as a solid. It is recrystallized from ethyl acetate/ether leaving 1.32 g (59%) of 4-(4-acetyl-2-methylphenoxy)butanoic acid as white crystals: m.p. 114-16° C; IR (KBr) 1730, 1650 cm-^{1}; ^{1}H-NMR ($CDCl_3$) is consistent with the desired product; MS (FAB) m/e 237 (M^+ + H). Anal. calc'd. for $C_{13}H_{16}O_4$: C, 66.08; H, 6.83; O, 27.09. Found: C, 65.88; H, 6.92; O, 27.20.

PREPARATION 7, COMPOUND **11**

4-(4-Formyl-2-methoxyphenoxy)butanoic acid

[0082] 4-Hydroxy-3-methoxybenzyl alcohol (1 g, 6.49 mmol) is treated with 1.73 g (7.13 mmol) of ethyl 4-bromobutyrate, 2.69 g (19.46 mmol) of potassium carbonate and a catalytic amount (97.22 mg 0.65 mmol) of sodium iodide as described in Preparation 3 to give 821 mg of 4-[4-(hydroxymethyl)-2-methoxyphenoxy]butanoic acid, ethyl ester as a light brown oil (47%). The ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (neat) 3500, 1730, 1620, 1600 cm-^{1}; MS (CI (low res)) m/e 269 (M+H), 251, 223, 195.

[0083] 4-[4-(Hydroxymethyl)-2-methoxyphenoxy]butanoic acid, ethyl ester (431 mg, 1.61 mmol) is treated with 1.0 g (4.8 mmol) of pyridinium chlorochromate by the procedure described in Example 5 to give 280 mg (65%) of 4-(4-formyl-

2-methoxyphenoxy)butanoic, ethyl ester as a colorless oil. The ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (neat) 1730, 1690, 1600, 1580 cm^{-1}.

[0084] 4-(4-Formyl-2-methoxyphenoxy)butanoic acid, ethyl ester (240 mg, 0.90 mmol) is dissolved in 3 mL of methanol/water (3:2) and treated with 435 mg (3.15 mmol) of potassium carbonate according to the procedure described for Example 4 to give 125 mg (58%) of 4-(4-formyl-2-methoxyphenoxy)butanoic acid as a white powder: m.p. 143-148°; the ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (KBr) 3575, 3500, 1720, 1700, 1680, 1600, 1585 cm-^{1}; MS (CI (low res)) m/e 239 (M+H), 221, 207, 153.

PREPARATION 8, COMPOUND **12**

4-Formylbenzenepropanoic acid

[0085] A mixture of 253 mg (1.44 mmol) of 4-formylcinnamic acid and 32.61 mg of platinum oxide in 10 mL of methanol is stirred overnight at room temperature under an atmosphere of hydrogen supplied by a balloon. The mixture is filtered through celite and concentrated *in vacuo.* The residue is dissolved in 0.1N sodium hydroxide solution and washed with ether. The aqueous layer is then acidified and the product is extracted with ethyl acetate. The organic layer is washed with saturated sodium chloride solution and dried over magnesium sulfate. The solvent is removed *in vacuo* to afford an inseparable mixture of 4-formylphenylpropanoic acid and other reduction products. The mixture is utilized in the next reaction without characterization or further purification.

PREPARATION 9, COMPOUND **13**

4-(2,3-Dimethoxy-5-formylphenoxy)butanoic acid

[0086] Employing the method of Preparation 3, 3.30 g (18.41 mmol) of 3,4-dimethoxy-5-hydroxybenzaldehyde is alkylated with ethyl 4-bromobutyrate. 4-(2,3-Dimethoxy-5-formylphenoxy)butanoic acid, ethyl ester is obtained as a yellow-orange oil after drying under high vacuum at 60° C (5.45 g, 100%): IR (neat) 1735, 1690 cm-^{1}; ^{1}H-NMR ($CDCl_3$) is consistent with the desired product; MS (FAB) m/e 297 (M^+ + H). Anal. calc'd. for $C_{15}H_{20}O_6$: C, 60.80; H, 6.80; O, 32.40. Found: C, 60.51; H, 6.86; O, 32.63.

[0087] Following the procedure of Preparation 3, a sample of 4.70 g (15.86 mmol) of 4-(2,3-dimethoxy-5-formylphenoxy)butanoic acid, ethyl ester is saponified giving the desired compound as a cream colored solid. This is recrystallized from ethyl acetate/ether, leaving 3.65 g (86%) of 4-(2,3-dimethoxy-5-formylphenoxy)butanoic acid as off-white crystals: m.p. 90-92° C; IR (KBr) 1710, 1690 cm-^{1}; ^{1}H-NMR ($CDCl_3$) is consistent with the desired product; MS (FAB) m/e 269 (M^+ + H). Anal. calc'd. for $C_{13}H_{16}O_6$: C, 58.20; H, 6.01; O, 35.79. Found: C, 58.10; H, 6.09; O, 35.81.

PREPARATION 10, COMPOUND **14**

4-(4-Acetyl-2,6-dimethoxyphenoxy)butanoic acid

[0088] Utilizing the procedure of Preparation 3, 2.61 g (13.32 mmol) of 4-acetyl-2,6-dimethoxyphenol is treated with ethyl 4-bromobutyrate. This gives the desired product after drying at -70° C, under high vacuum, as a brown oil. This is chromatographed on a column of silica gel, and eluted with a 1:1 mixture of ether/hexane leaving 0.40 g (10%) of 4-(4-acetyl-2,6-dimethoxyphenoxy)butanoic acid, ethyl ester as a colorless oil: IR (neat) 1735, 1675 cm-^{1}; ^{1}H-NMR ($CDCl_3$) is consistent with the desired product; MS (FAB) m/e 311.1489 Δ = +0.6 mmμ (M^+ + H). Anal. calc'd. for $C_{16}H_{22}O_6$: C, 61.92; H, 7.14; O, 30.94. Found: C, 61.48; H, 7.04; O, 31.48.

[0089] Following the method of Preparation 3, 0.179 g (0.577 mmol) of 4-(4-acetyl-2,6-dimethoxyphenoxy)-butanoic acid, ethyl ester is treated with potassium carbonate, producing an off-white solid. Recrystallization from ethyl acetate/hexane gives 4-(4-acetyl-2,6-dimethoxyphenoxy)butanoic acid as white crystals (0.14 g, 88%) : m.p. 122-24° C; IR (KBr) 1735, 1660 cm-^{1}; ^{1}H-NMR ($CDCl_3$) is consistent with the desired product; MS (FAB) m/e 283 (M^+ + H). Anal. calc'd. for $C_{14}H_{18}O_6$: C, 59.57; H, 6.43; O, 34.01. Found: C, 59.34; H, 6.40; O, 34.26.

PREPARATION 11, COMPOUND **15**

4-(4-Acetyl-2-methoxyphenoxy)butanoic acid.

[0090] Employing the procedure of Preparation 3, 2.21 g (13.32 mmol) of 4-hydroxy-3-methoxyacetophenone is alkylated, producing a solid. This is recrystallized as in Preparation 3, leaving 3.23 g (86%) of 4-(4-acetyl-2-methoxyphenoxy)butanoic acid, ethyl ester as white crystals: m.p. 53-55° C; IR (KBr) 1745, 1675 cm-^{1}; ^{1}H-NMR ($CDCl_3$) is

consistent with the desired product; MS (FAB) m/e 281 (M^+ + H). Anal. calc'd. for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19; 0, 28.54. Found: C, 64.26; H, 7.05; O, 28.69.

[0091] Following the method of Preparation 3, 2.74 g (9.78 mmol) of 4-(4-acetyl-2-methoxyphenoxy)butanoic acid, ethyl ester is saponified. This produces 4-(4-acetyl-2-methoxyphenoxy)butanoic acid as off-white crystals after recrystallization from ethyl acetate (1.61 g, 87%): m.p. 161-63° C; IR (KBr) 1720, 1670 cm-1; ^{1}H-NMR ($CDCl_3$) is consistent with the desired product; MS (FAB) m/e 253 (M^+ + H). Anal. calc'd. for $C_{13}H_{16}O_5$: C, 61.90; H, 6.39; O, 31.71. Found: C, 61.75; H, 6.37; O, 31.88.

PREPARATION 12, COMPOUND **16**

4-[4-(3-Oxobutyl)phenoxy]butanoic acid

[0092] 4-Hydroxybenzylacetone (2 g, 12.18 mmol) is treated with 2.61 g (13.4 mmol) of ethyl 4-bromobutyrate, 5.05 g (36.5 mmol) of potassium carbonate and a catalytic amount (182 mg 1.22 mmol) of sodium iodide in 2 mL N,N-dimethylformamide as described in Preparation 3 to give 2.73 g of 4-[4-(3-oxobutyl)phenoxy]-butanoic, ethyl ester as a light brown oil (80%): m.p. 32-34°; the ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (neat) 1730, 1720, 1610, 1580, 1510 cm-1; MS (CI (low res)) m/e 279 (M+H), 233, 221; Analysis calc'd for $C_{16}H_{22}O_4$: C, 69.04; H, 7.97; found: C, 68.33; H, 7.68.

[0093] 4-[4-(3-Oxobutyl)phenoxy]butanoic acid, ethyl ester (716 mg, 2.57 mmol) is dissolved in 5 mL of methanol/water (3:2) and treated with 1.24 g (9.0 mmol) of potassium carbonate according to the procedure described for Example 4 to give 385 mg (60%) of 4-[4-(3-oxobutyl)phenoxy]butanoic acid as a white powder: m.p. 97-99°; the ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (neat) 1730, 1700, 1620, 1520 cm^{-1}; Analysis calc'd for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25; found: C, 66.55; H, 7.09.

EXAMPLE 13, COMPOUND **17**

4-(2-Acetyl-5-methoxyphenoxy)butanoic acid

[0094] Following the procedure of Preparation 3, 2.21 g (13.32 mmol) of 2-hydroxy-4-methoxyacetophenone is alkylated and worked up as before to leave 3.40 g (91%) of 4-(2-acetyl-5-methoxyphenoxy)butanoic acid, ethyl ester as a yellow oil: IR (neat) 1740, 1665 cm-1; ^{1}H-NMR ($CDCl_3$) is consistent with the desired product; MS (FAB) m/e 281 (M^+ + H). Anal. calc'd. for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19; O, 28.54. Found: C, 64.06; H, 7.24; O, 28.70.

[0095] Utilizing the method of Preparation 3, 2.50 g (8.92 mmol) of 4-(2-acetyl-5-methoxyphenoxy)butanoic acid, ethyl ester is treated with potassium carbonate, producing a white solid. This is recrystallized from ethyl acetate/ether leaving 1.61 g (71%) of 4-(2-acetyl-5-methoxyphenoxy)butanoic acid as colorless crystals: m.p. 127-29° C; IR (KBr) 1720, 1655 cm-1; ^{1}H-NMR ($CDCl_3$) is consistent with the desired product; MS (FAB) m/e 253 (M^+ + H). Anal. calc'd. for $C_{13}H_{16}O_5$: C, 61.90; H, 6.39; O, 31.71. Found: C, 61.82; H, 6.37; O, 31.81.

PREPARATION 14, COMPOUND **18**

4-[4-(3-Oxopropyl)phenoxy]butanoic acid

[0096] Following the procedure of Preparation 3, 2.80 g (18.41 mmol) of 3-(4-hydroxyphenyl-1-propanol) is alkylated with ethyl 4-bromobutyrate. The product is dried at 70° C, under high vacuum, leaving 4.70 g (96%) of 4-[4-(3-hydroxypropyl)phenoxy]butanoic acid, ethyl ester as a colorless oil: IR (neat) 3400 (br.), 1735 cm-1; ^{1}H-NMR ($CDCl_3$) is consistent with the desired product; MS (CI) m/e 267 (M^+ + H). Anal. calc'd. for $C_{15}H_{22}O_4$: C, 67.65; H, 8.33; O, 24.03. Found: C, 67.40; H, 8.20; O, 24.38.

[0097] After the method of Preparation 3, 4.13 g (15.51 mmol) of 4-[4-(3-hydroxypropyl)phenoxy]butanoic acid, ethyl ester is saponified with potassium carbonate to produce a solid. This is recrystallized from an ethyl acetate-hexane mixture giving 2.45 (66%) of 4-[4-(3-hydroxypropyl)phenoxy]butanoic acid as white crystals: m.p. 92-94° C; IR (KBr) 3420 (br.), 1710 cm-1; ^{1}H-NMR ($CDCl_3$) is consistent with the desired product; MS (CI) m/e 239 (M^+ + H). Anal. calc'd. for $C_{13}H_{18}O_4$: C, 65.53; H, 7.61; O, 26.86. Found: C, 65.75; H, 7.87; O, 26.38.

[0098] A 1.19 g (5.00 mmol) sample of 4-[4-(3-hydroxypropyl)phenoxy)butanoic acid is dissolved with stirring in 250 mL of methylene dichloride. Next, 3.77 g (17.49 mmol) of pyridinium chlorochromate is added, the mixture is stirred for 4 hr, and then filtered through a celite pad. The reaction mixture is then diluted with an equal volume of ether, precipitating out salts. This mixture is then filtered through a silica gel pad, and the filtrate evaporated, giving a brown solid. The solid is recrystallized from an ether-hexane mixture producing 0.21 (18%) of 4-[4-(3-oxopropyl)phenoxy] butanoic acid as off-white crystals: m.p. 100-03° C; IR (KBr) 1715 cm-1; ^{1}H-NMR ($CDCl_3$) is consistent with the desired

product; MS (CI) m/e 237 (M^+ + H). Anal. calc'd. for $C_{13}H_{16}O_4$: C, 66.09; H, 6.83; O, 27.09. Found: C, 65.91; H, 6.72; O, 27.35.

EXAMPLE 15, COMPOUND **20**

4-[(2-Acetyl-1-naphthalenyl)oxy]butanoic acid

[0099] A 3.42 g (18.37 mmol) sample of 1-hydroxy-2-acetonapthone is alkylated as in Preparation 3. The crude product is dried under high vacuum at 60° C to give 5.21 g (94%) of 4-[(2-acetyl-1-naphthalenyl)-oxy]butanoic acid, ethyl ester as a golden liquid: IR (neat) 1730, 1665 cm^{-1}; ^{1}HNMR ($CDCl_3$) is consistent with the desired product; MS (FAB) m/e 301(M^+ + H). Anal. calc'd. for $C_{18}H_{20}O_4$: C, 71.98; H, 6.71; O, 21.31. Found: C, 72.11; H, 6.58; O, 21.31.

[0100] Utilizing the method of Preparation 3, 2.84 g (9.46 mmol) of 4-[(2-acetyl-1-naphchalenyl)oxy]butanoic acid, ethyl ester is saponified. The crude product is recrystallized from ethyl acetate/ether to give 1.15 g (45%) of 4-[(2-acetyl-1-naphthalenyl)oxy]butanoic acid as golden crystals: m.p. 104-06° C; IR (KBr) 1720, 1640 cm^{-1}; ^{1}HNMR ($CDCl_3$) is consistent with the desired product; MS (FAB) m/e 273 (M^+ + H). Anal. calc'd. for $C_{16}H_{16}O_4$: C, 70.58; H, 5.92; O, 23.50. Found: C, 70.40; H, 5.89; O, 23.71.

PREPARATION 16, COMPOUND **21**

4-[4-(4-Fluorobenzoyl)phenoxy]butanoic acid

[0101] Following the method of Preparation 3, 3.98 g (18.41 mmol) of 4-fluoro-4'-hydroxybenzophenone is alkylated with ethyl 4-bromobutyrate. The crude yellow solid product is recrystallized from ether providing 2.97 g (49%) of 4-[4-(4-fluorobenzoyl)phenoxy]butanoic acid, ethyl ester as white crystals: m.p. 57-59° C; IR (KBr) 1735, 1645 cm^{-1}; ^{1}HNMR ($CDCl_3$) is consistent with the desired product; MS (FAB) m/e 311 (M^+ + H). Anal. calc'd. for $C_{19}H_{19}O_4F$: C, 69.08; H, 5.80; F, 5.75; O, 19.37. Found: C, 69.09; H, 5.62; F, 5.95; O, 19.34.

[0102] Utilizing the procedure of Preparation 3, 0.48 g (1.45 mmol) of 4-[4-fluorobenzoyl)phenoxy]butanoic acid, ethyl ester is saponified. The crude white solid product is recrystallized from an ether-hexane mixture leaving 0.16 g (36%) of 4-[4-(4-fluorobenzoyl)phenoxy]-butanoic acid as white crystals: m.p. 109-111° C; IR (KBr) 1735, 1700 1640 cm^{-1}; ^{1}HNMR ($CDCl_3$) is consistent with the desired product; MS (FAB) m/e 303 (M^+ + H). Anal. calc'd. for $C_{17}H_{15}O_4F$: C, 67.54; H, 5.00; F, 6.28; O, 21.18. Found: C, 67.28; H, 4.89; F, 6.41; O, 21.42.

EXAMPLE 17, COMPOUND **22**

4-(4-Acetylphenyl)-1-piperazinevaleric acid

[0103] 4'-Piperazinoacetophenone (102 mg) is dissolved in 1 mL of N,N-dimethylformamide. After addition of methyl 5-bromovalerate (0.077 mL) and potassium carbonate (69 mg), the mixture is stirred at room temperature for 65 hr. TLC (10% MeOH/CH_2Cl_2) should show a single product spot without residual starting material. The reaction solution is evaporated under vacuum. The residue is taken up in methylene chloride, washed twice with water and dried over sodium sulfate. Evaporation of the solvent yields 137 mg of 4-(4-acetylphenyl)-1-piperazinevaleric acid, methyl ester as yellow crystals whose ^{1}H-NMR ($CDCl_3$) spectrum is consistent with the assigned structure.

[0104] 4-(4-Acetylphenyl)-1-piperazinevaleric acid, methyl ester (15.3 mg) is suspended in 0.1 mL of potassium hydroxide solution (33.2 mg/mL). After heating at 100° C for 150 min, the starting material is completely dissolved and absent by TLC (10% MeOH/CH_2Cl_2). After acidifying the reaction solution to pH 4 by adding 0.2 N HCl, the aqueous solution is extracted with methylene chloride. After evaporation of the organic layer to dryness, the residue is dissolved in methylene chloride and filtered. Evaporation of the organic layer gives 7 mg of 4-(4-acetylphenyl)-1-piperazinevaleric acid as a white solid. ^{1}H-NMR ($CDCl_3$) spectrum is consistant with the assigned structure. MS (FAB) m/e 305 (M^+ + H), 327 (M^+ + Na), 348 (M^+ + 2Na-H).

PREPARATION 18, COMPOUND **25**

4-(2-Chloro-4-formylphenoxy)butanoic acid

[0105] Following the procedure of Preparation 3, 2.88 g (18.41 mmol) of 3-chloro-4-hydroxybenzaldehyde is alkylated as before. This produces 4.65 g (93%) of 4-(2-chloro-4-formylphenoxy)butanoic acid, ethyl ester as an orange oil: IR (neat) 1730, 1685 cm^{-1}; ^{1}HNMR ($CDCl_3$) is consistent with the desired product; MS (CI) m/e 271 (M^+ + H). Anal. calc'd. for $C_{13}H_{15}O_4Cl$: C, 57.68; H, 5.58; Cl, 13.10; O, 23.64. Found: C, 58.05; H, 5.37; Cl, 12.43; O, 24.15.

[0106] After the method of Preparation 3, 3.52 g (13.00 mmol) of 4(2-chloro-4-formylphenoxy)butanoic acid, ethyl ester is saponified to give a white solid. This is recrystallized from ethyl acetate resulting in 1.78 g (56%). of 4-(2-chloro-4-formylphenoxy)butanoic acid as white crystals: m.p. 128-31° C; IR (KBr) 1730, 1650 cm^{-1}; ^{1}HNMR ($CDCl_3$) is consistent with the desired product; MS (CI) m/e 243 (M^+ + H). Anal. calc'd. for $C_{11}H_{11}O_4Cl$: C, 54.45; H, 4.57; Cl, 14.61; O, 26.37. Found: C, 54.61; H, 4.70; Cl, 14.25; O, 26.42.

EXAMPLE 19, COMPOUND **26**

5-Acetyl-2-(3-carboxypropoxy)benzoic acid, methyl ester

[0107] Under dry condition, 3.58 g (18.41 mmol) of 5-acetylsalicylic acid, methyl ester is dissolved in 25 mL of dry N,N-dimethylformamide. To this solution is added 3.07 g (20.58 mmol) of 5-bromo-1-pentene, 6.83 (20.58 mmol) of potassium carbonate, and 0.246 g (1.65 mmol) of potassium iodide, and the reaction mixture is stirred for 24 hr at ambient temperature. Another portion of 5-bromopentene is added to the reaction, followed by one-half portions of the other two reagents above, and stirring is continued for 72 hr. The mixture is then evaporated under high vacuum at 70° C. The residue is partitioned between ether/water and the organic phase is separated, dried with magnesium sulfate, filtered, and evaporated under vacuum to leave 4.60 g (95%) of 5-acetyl-2-(4-pentenyloxy)benzoic acid, methyl ester as a yellow liquid: IR (neat) 1735, 1710, 1680 cm^{-1}; ^{1}HNMR ($CDCl_3$) is consistent with the desired product; MS (FAB) m/e 263 (M^+ + H). Anal. calc'd. for $C_{15}H_{18}O_4$: C, 68.69; H, 6.92; O, 24.40. Found: C, 68.60; H, 6.92; O, 24.46.

[0108] A sample of 0.203 g (0.775 mmol) of 5-acetyl-2-(4-pentenyloxy)benzoic acid, methyl ester is dissolved in 5 mL of methylene dichloride, under an argon atmosphere, and cooled to -78° C in a dry ice acetone bath, with stirring. Next, ozone gas is passed through this solution for 10 min, until it turns a light bluish color. Then 0.5 mL of dimethyl sulfide is added to quench the reaction and it is allowed to warm to room temperature for 2 hr. The mixture is then evaporated under high vacuum, leaving the crude aldehyde product as an oil which is used "as is" for the second step. It is dissolved in 5 mL of N,N-dimethylformamide, and 1.02 g (2.71 mmol) of pyridinium dichromate is added. This reaction mixture is sealed and allowed to stand for 20 hr. It is next poured into 50 mL of water, extracted with ether, and the organic phase is washed with water again, dried with magnesium sulfate, filtered, and evaporated, which gives oily crystals. These are recrystallized from a mixture of ethyl acetate and hexane, producing 0.109 g (50%) of 5-acetyl-2-(3-carboxypropoxy)benzoic acid, methyl ester as white crystals: m.p. 111-113° C; IR (KBr) 1725, 1645 cm^{-1}; ^{1}HNMR ($CDCl_3$) is consistent with the desired product; MS (FAB) m/e 281 (M^+ + H). Anal. calc'd. for $C_{14}H_{16}O_6$: C, 60.00; H, 5.75; O, 34.25. Found: C, 59.96; H, 5.75; O, 34.27.

PREPARATION 20, COMPOUND **27**

4-(4-Formyl-2-nitrophenoxy)butanoic acid

[0109] 4-Hydroxy-3-nitrobenzyl alcohol (1 g, 5.91 mmol) is treated with 1.44 g (7.39 mmol) of ethyl 4-bromobutyrate, 2.86 g (20.69 mmol) of potassium carbonate and a catalytic amount (88 mg .59 mmol) of sodium iodide as described in Preparation 3 to give 1.45 g of 4-[4-(hydroxymethyl)2-nitrophenoxy]butanoic acid, ethyl ester as a light yellow oil (86%). The ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (neat) 3400, 1730, 1710, 1630, 1580 cm^{-1}; MS (CI) m/e 284 (M+H), 238; Analysis calc'd for $C_{13}H_{17}O_6N$: C, 55.12; H, 6.05; found: C, 55.36; H, 6.03.

[0110] 4-[4-(Hydroxymethyl)2-nitrophenoxy]butanoic acid, ethyl ester (300 mg, 1.06 mmol) is treated with 799 mg (3.71 mmol) of pyridinium chlorochromate by the procedure described in Example 5 to give 188 mg (63%) of 4-(4-formyl-2-nitrophenoxy)butanoic acid, ethyl ester as a colorless oil. The ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (neat) 1730, 1700, 1610, 1570 cm^{-1}; MS (CI) m/e 282 (M+H).

[0111] 4-(4-Formyl-2-nitrophenoxy)butanoic acid, ethyl ester (135 mg, 0.48 mmol) is dissolved in 3 mL of methanol/water (3:2) and treated with 232 mg (1.68 mmol) of potassium carbonate according to the procedure described for Example 4 to give 84 mg (69%) of 4-(4-formyl-2-nitrophenoxy)butanoic acid as a yellow powder: m.p. 136-139°; the ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (KBr) 3400, 1730, 1700, 1650, 1600, 1570 cm^{-1}; MS (CI) m/e 254, 236, 224, 208, 196, 168.

EXAMPLE 21, COMPOUND **28**

4-[2-[[(4-Acetylphenyl)amino]methyl]-6-methoxyphenoxy]butanoic acid

[0112] 4'-(2-Hydroxy-3-methoxybenzylamino)acetophenone (500 mg, 1.84 mmol) is treated with 629 mg (3.22 mmol) of ethyl 4-bromobutyrate, 764 mg (5.53 mmol) of potassium carbonate and a catalytic amount (182 mg, 1.22 mmol) of sodium iodide in 2 mL N,N-dimethylformamide as described in Preparation 3 to give 680 mg of 4-[2-[[(4-acetylphenyl)

amino]methyl]-6-methoxyphenoxy]butanoic acid, ethyl ester as a light brown oil (95%). The ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (neat) 3400, 1730, 1660, 1600 cm^{-1}; MS (CI) m/e 386 (M+H), 115; Analysis calc'd for $C_{22}H_{27}O_5N$: C, 68.55; H, 7.06; N, 3.63; found: C, 68.27; H, 6.81; N, 3.54.

[0113] 4-[2-[[(4-Acetylphenyl)amino]methyl]-6-methoxy-phenoxy]butanoic acid, ethyl ester (250 mg, 0.65 mmol) is. dissolved in 5 mL of methanol/water (3:2) and treated with 313 mg (2.27 mmol) of potassium carbonate according to the procedure described for Example 4 to give 166 mg (71%) of 4-[2-[[(4-acetylphenyl)amino]-methyl]-6-methoxy-phenoxy]butanoic acid as a red colored solid: m.p. 85-95° C; The ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (KBr) 3400, 1720, 1630, 1580 cm^{-1}; MS (CI) m/e calc'd for $C_{20}H_{23}O_5NNa$: 380.1473, found 380.1482; 358 (M+H), 233, 223, 221, 136.

EXAMPLE 22, COMPOUND **29**

5-Acetyl-2-(3-carboxypropoxy)-benzoic acid, 1-(3-bromopropyl) ester

[0114] To a solution of 0.744 g (3.00 mmol) of 5-acetyl-2-(4-pentenyloxy)-benzoic acid (Example 19), under an argon atmosphere, with stirring, in 36 mL of methylene dichloride, is added 1.67 g (12.0 mmol) of 3-bromopropanol. This is followed by 0.912 g (9.0 mmol) of triethyl amine and by 1.66 g (3.75 mmol) of benzotriazol-1-yloxytris(dimethylamino) phosphonium hexafluorophosphate and the reaction is stirred for 20 hr. The mixture is then evaporated, under high vacuum at 65° C. The residue is partitioned between ether and water, the ether phase is washed twice more with water, dried with magnesium sulfate, filtered, and evaporated leaving a gum. This is chromatographed on a column of silica gel, and eluted with ethyl acetate/hexane (1:2) to give 0.80 g (72%) of 5-acetyl-2-(4-pentenyloxy)-benzoic acid, 1-(3-bromopropyl) ester as a colorless oil: IR (neat) 1730, 1700, 1680 cm^{-1}; ^{1}HNMR ($CDCl_3$) is consistent with the desired product; MS (FAB) m/e 369 (M^+ + H). Anal. calc'd. for $C_{17}H_{21}O_4Br$: C, 55.30; H, 5.73; O, 17.33; Br 21.64. Found: C, 55.34; H, 5.44; O, 17.34; Br 21.88.

[0115] Following the procedure of Example 19, 0.377 g (1.02 mmol) of 5-acetyl-2-(4-pentenyloxy)-benzoic acid, 1-(3-bromopropyl) ester is ozonized, and then further oxidized with pyridinium dichromate producing a colorless gum which partially crystallizes. This is recrystallized from a mixture of equal parts of ethyl acetate and hexane leaving 0.277 g (70%) of 5-acetyl-2-(3-carboxypropoxy)-benzoic acid, 1-(3-bromopropyl) ester as white crystals: m.p. 103-05° C; IR (KBr) 1730, 1645 cm^{-1}; ^{1}HNMR ($CDCl_3$) is consistent with the title product; MS (CI) m/e 389 (M^+ + H). Anal. calc'd. for $C_{16}H_{19}O_6Br$: C, 49.63; H, 4.95; O, 24.79; Br, 20.63. Found: C, 49.90; H, 4.75; O, 24.94; Br, 20.39.

PREPARATION 23, COMPOUND **30**

4-(4-Acetyl-3-fluorophenoxy)butanoic acid

[0116] A solution of 2-fluoro-4-methoxyacetophenone in 5 mL of DMSO is stirred at 100° C in the presence of 730 mg (15 mmol) of sodium cyanide to give a dark viscous sludge. The mixture is allowed to cool, then poured into 50 mL of ice water and acidified with 6N aqueous HCl. The acidic solution is extracted with' ethyl acetate (50 mL x 2) and the organic layers are combined and washed with water. The organic layer is then extracted twice with 1.0 N aqueous sodium hydroxide solution. The basic layer is washed once with ether, then acidified with solid sodium bisulfate and extracted with ethyl acetate twice. The ethyl acetate layers are combined, then washed with 10% sodium bisulfate solution and saturated sodium chloride solution. The organic phase is dried over magnesium sulfate and concentrated *in vacuo* at ambient temperature to give 143 mg (31%) of an oil.

[0117] The oil isolated above is dissolved in 1 mL of N,N-dimethylformamide and treated with 205 mg (1.05 mmol) of ethyl 4-bromobutyrate, 4.07 g (2.95 mmol) of potassium carbonate and a catalytic amount (1.26 mg, 0.008 mmol) of sodium iodide according to the procedure described in Preparation 3 to give 39 g of 4-(4-acetyl-3-fluorophenoxy) butanoic acid, ethyl ester as a light brown oil (17%). The ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; MS (CI (low res)) m/e calc'd for $C_{14}H_{18}O_4F$: 269.1189, found 269.1191.

[0118] 4-(4-Acetyl-3-fluorophenoxy)butanoic acid, ethyl ester (20 mg, 0.0745 mmol) is dissolved in 1 mL of methanol/ water (3:2) and treated with 30.91 mg (0.22 mmol) of potassium.carbonate according to the procedure described for Example 4 to give 14 mg (82%) of 4-(4-acetyl-3-fluorophenoxy)butanoic acid as a white powder: m.p. 110-111° C; The ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (KBr) 1710, 1670, 1610 cm^{-1}; MS m/e calc'd for $C_{12}H_{13}O_4FNa$: 263.0695, found 263.0699.

EXAMPLE 24, COMPOUND **31**

(2-Acetylphenoxy)butanoic acid

[0119] 2-Acetylphenol (1 g, 7.34 mmol) is treated with 1.79 g (9.18 mmol) of ethyl 4-bromobutyrate, 3.55 g (25.71 mmol) of potassium carbonate and a catalytic amount (11 mg, 0.07 mmol) of sodium iodide as described in Preparation 3 to give 1.84 g of (2-acetylphenoxy)-butyric acid, ethyl ester as a light yellow oil which solidified upon standing: m.p. 43-45° C; The ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (neat) 1730, 1660, 1600 cm^{-1}; MS (CI) m/e 251 (M+H), 232, 205.

[0120] (2-Acetylphenoxy)butanoic acid, ethyl ester (500 mg, 2.00 mmol) is dissolved in 3 mL of methanol/water (3:2) and treated with 828 mg (5.99 mmol) of potassium carbonate according to the procedure described for Example 4 to give 412 mg (93%) of (2-acetylphenoxy)bucanoic acid as a white powder. The ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (KBr) 1710, 1670, 1590 cm-^{1}; MS m/e calc'd for $C_{12}H_{15}O_4$: 223.0970, found 223.0971.

EXAMPLE 25, COMPOUND **32**

2-Acetyl-10H-phenothiazine-10-hexanoic acid

[0121] A solution of 500 mg (2.07 mmol) of 2-acetylphenothiazine in 8 mL of tetrahydrofuran is cooled to -78° C and 4.14 mL (2.07 mmol) of a 0.5 M solution of potassium bis(trimethylsilyl)amide in toluene is added. After five minutes, a solution of [(6-bromohexyl)oxy]-(1,1-dimethylethyl)dimethyl silane in 2 mL of tetrahydrofuran is added and the reaction is allowed to warm to room temperature. The mixture is diluted with 25 mL of ethyl acetate and washed with 10% sodium bisulfate solution and saturated sodium chloride solution, then dried over magnesium sulfate and concentrated *in vacuo* to give a dark colored residue. Flash chromatography (3:1 hexane/ethyl acetate) provides 318 mg (33%) of 1-[10-[6-[[(1,1-dimethylethyl)dimethylsilyl]oxy]hexyl]-10H-phenothiazin-2-yl]ethanone as a dark colored oil. The ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (neat) 1680, 1600, 1560 cm^{-1}; MS m/e calc'd for $C_{26}H_{37}NO_2SSi$: 455.2314, found 455.2312.

[0122] A solution of 150 mg (0.33 mmol) of 1-[10-[6-[[(1,1-dimethylethyl)dimethylsilyl]oxy]hexyl]-10H-phenothiazin-2-yl]ethanone in 0.6 mL of tetrahydrofuran is treated with 0.41 mL (0.41 mmol) of 1 M tetrabutylammonium fluoride in tetrahydrofuran. The reaction is stirred for 3 hr at room temperature, then diluted with 20 mL of ethyl acetate. The organic layer is washed successively with 10% sodium bisulfate solution and saturated sodium chloride solution, then dried over magnesium sulfate and concentrated *in vacuo* to give 114 mg of 1-[10-(6-hydroxyhexyl)-10H-phenothiazin-2-yl]ethanone as a dark oil. The ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product; IR (neat) 3400, 1680, 1590, 1560 cm-^{1}; MS m/e calc'd for $C_{20}H_{23}NO_2S$: 341.1449, found 341.1456.

[0123] A solution of 41 mg (0.12 mmol) of 1-[10-(6-hydroxyhexyl)-10H-phenothiazin-2-yl]ethanone in 0.16 mL of N,N-dimethylformamide is treated with 158 mg (0.42 mmol) of pyridinium dichromate and stirred at room temperature for 12 hr. The mixture is diluted with ether and filtered through a pad of celite with the aid of 100 mL of ether. The filtrate is washed successively with 10% sodium bisulfate solution and saturated sodium chloride solution, then dried over magnesium sulfate and concentrated in *vacuo* to give 10 mg (23%) of 2-acetyl-10H-phenothiazine-10-hexanoic acid as a dark residue. The ^{1}H NMR (300 MHz, $CDCl_3$) is consistent with the desired product. MS (CI) m/e 323 (M^+ + H).

EXAMPLE 26, COMPOUND **34**

5-Acetyl-2-(3-carboxypropoxy)-N-(2-dimethylaminoethyl)-benzamide

[0124] A sample of 0.140 g (0.50 mmol) of 5-acetyl-2-(3-carboxypropoxy)-benzoic acid, methyl ester (Example 19) is heated on a steam bath, under dry conditions with 5.49 mL (50.0 mmol) of N,N-dimethylethylenediamine for 5 hr. The mixture is allowed to cool for 20 hr to ambient temperature and evaporated under vacuum at 55° C. The brown gum produced is triturated with ether, and the remaining residue taken up in water and acidified with hydrochloric acid. This is then extracted with ethyl acetate, and the aqueous solution is evaporated under vacuum, leaving a gum. It is next triturated with hot chloroform and this solution is evaporated to give a brown glass. This is chromatographed on a preparatory silica gel plate which is eluted with a 9/1 mixture of chloroform to methanol. The product band is cut from the plate, triturated with the above solvent mixture, filtered, and evaporated leaving 0.025 g (15%) of 5-acetyl-2-(3-carboxypropoxy)-N-(2-dimethylaminoethyl)-benzamide as a light brown gum: MS (FAB) m/e 337.1753 Δ = +0.9 mmμ (M^+ + H), 359 (M^+ + Na). ^{1}HNMR ($CDCl_3$) is consistent with the desired product.

EXAMPLE 27, COMPOUND **35**

5-Acetyl-2-(3-carboxypropoxy)-N-(2-trimethylaminoethyl)-benzamide, internal salt

[0125] To 100 mg of 5-acetyl-2-(3-carboxypropoxy)-N-(2-dimethylaminoethyl)-benzamide in 2 mL of methanol and 8 mL of pH 8.6 phosphate buffer is added 0.5 mL of dimethyl sulfate. The reaction pH is monitored about every 30 min and 0.1 N, sodium hydroxide is added as needed to return the pH to -8.5. After 4 hr the solvents are removed under vacuum and the product is purified on BioSil A with a methanol-in-chloroform gradient to give 5-acetyl-2-(3-carbomethoxypropoxy) -N-(2-trimethylaminoethyl)-benzamide, chloride which is taken on to the next step. ^{1}H-NMR ($CDCl_3$): 8.6 ppm (1H, d), 8.1 ppm (1H, dd), 7.1 ppm (1H, d), 4.3 ppm (2H, t), 4.0 ppm (2H, br t), 3.9 ppm (2H, br s), 3.7 ppm (3H, s), 3.7 ppm (1H, t), 3.3 ppm (9H, s), 2.1 ppm (3H, s), 2.1 ppm (2H, t), 2.3 ppm (2H, m) .

[0126] The above product is dissolved in 2 mL of tetrahydrofuran and treated with an excess of 1 N sodium hydroxide for 16 hr at ambient temperature. The organic cosolvent is removed under vacuum and the aqueous solution which remains is acidified with 1 N HCl to a pH of about 5. The solution is then evaporated under vacuum to give a glass which crystallizes on standing. The resultant 5-acetyl-2- (3-carboxypropoxy) -N-(2-trimethylaminoethyl)benzamide, internal salt can be used without further purification. MS (FAB) m/e 351 (M^+ + H).

EXAMPLE 28, COMPOUND **36**

5-Acetyl-2-[N-(2-dimethylaminoethyl)-3-carboxamidopropoxy]benzoic acid, internal salt

[0127] To 1.16 g of 5-acetylsalicylic acid, methyl ester in 10 mL of N,N-dimethylformamide is added 1 g of chloroacetic acid, methyl ester and 1.2 g of potassium carbonate. After stirring this mixture at ambient temperature for 16 hr the reaction is filtered, diluted with ethyl acetate, and washed once with water and twice with brine. The ethyl acetate is dried with magnesium sulfate, filtered, and evaporated to give 5-acetyl-2-(carboxymethoxy)benzoic acid as a crude product. Crystallization from methanol at -15° C gives 0.6 g of white crystals. ^{1}H-NMR ($CDCl_3$): 8.5 ppm (1H, d), 8.1 ppm (1H, dd), 6.9 ppm (1H, d), 4.8 ppm (2H, s), 4.0 ppm (3H, s), 3.8 ppm (3H, s), 2.6 ppm (3H, s).

[0128] 450 mg of the above product is stirred in 1 mL of N,N-dimethylethylenediamine at ambient temperature for 16 hr. The reaction is then diluted with ethyl acetate and water. The water layer is extracted five times with ethyl acetate and the ethyl acetate from the various extractions is pooled, dried with magnesium sulfate, filtered, and evaporated to give 380 mg of 5-acetyl-2-[N-(2-dimethylaminoethyl)-3-carboxamidopropoxy]benzoic acid , methyl ester as a yellowish oil which is pure enough for further use. ^{1}H-NMR ($CDCl_3$): 8.6 ppm (1H, d), 8.2 ppm (1H, dd), 8.1 ppm (1H, br t), 7.0 ppm (1H, d), 4.7 ppm (2H, s), 4.0 ppm (3H, s), 3.5 ppm (2H, q), 2.7 ppm (3H, s), 2.6 ppm (2H, t), 2.3 ppm (6H, s).

[0129] To 280 mg of the above compound in 15 mL of methanol and 5 mL of chloroform is added 1 mL of methyl iodide. After 3 hr at ambient temperature the volatile components are removed. 1H-NMR indicates the presence of the desired 5-acetyl-2-[N-(2-trimethylaminoethyl)-3-carboxamidopropoxy]benzole acid, methyl ester, iodide. ^{1}H-NMR ($CDCl_3$+CD_3OD): 8.8 ppm (1H, br t), 8.6 ppm (1H, d), 8.2 ppm (1H, dd), 7.1 ppm (1H, d), 4.7 ppm (2H, s), 4.0 ppm (3H, s), 3.9 ppm (2H, q), 3.8 ppm (2H, t), 3.4 (9H, s), 2.6 ppm (3H, s).

[0130] The above compound is dissolved in - 5 mL of methanol. Five equivalents of sodium hydroxide is added as a 5N solution in water. After 5 hr at ambient temperature the pH is adjusted to -7.5 with dilute HCl and the volatile components are removed under vacuum to give a crude product containing 5-acetyl-2-[N-(2-trimethylaminoethyl)-3-carboxamidopropoxy)benzoic acid, internal salt. MS (CI) m/e 323 (M^+ + H).

SYNTHESIS OF STRUCTURES B (Scheme 1)

General Procedure

[0131] The drug-hydrazide derivative (Q-Sp-S-S-W wherein Q = H_2NHN-) is dissolved in alcohol or other compatible organic solvent containing ~3 to 10 equivalents of the carbonyl linker and ~1-10% acetic acid or other appropriate acid catalyst. A minimal amount of solvent gives a faster reaction. Anhydrous conditions give the best results as the condensation is an equilibrium reaction. The reaction is allowed to proceed at a temperature of ~20-60° C until complete by HPLC or alternately by TLC. This requires from a. few hours to a day or more depending on the linker and the specific reaction conditions. The solvents are removed *in vacuo* and the crude product is purified on an appropriate silica gel, such as Biosil-A™, using an appropriate solvent system, such as a gradient of 0 to 20% methanol in either chloroform or ethyl acetate. The products are sufficiently pure for subsequent steps.

EXAMPLE 29

[0132] 4-Formylphenoxyacetic acid (**1**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 4.4 min,
FAB MS: 1641 (M+H),
UV max at 291 and 305 nm (acetate),
^{1}H-NMR: See Fig. I.

PREPARATION 30

[0133] 4-Formylbenzoic acid (**2**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 5.2 min,
FAB MS: 1611 (M+H),
UV max at 292 and 302 nm (ethanol),
^{1}H-NMR: See Fig. II.

PREPARATION 31

[0134] 4-Formyl-3-methoxyphenoxyacetic acid (**3**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 4.7 min,
FAB MS: 1671 (M+H),
UV max at 282, 291, and 325 nm (echanol),
^{1}H-NMR: See Fig. III.

PREPARATION 32

[0135] 6-Formyl-2-naphthoic acid (**4**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 6.1 min,
FAB MS: 1661 (M+H),
UV max at 257, 267, 277, 313, and 321 nm (ethanol),
^{1}H-NMR: See Fig. IV.

PREPARATION 33

[0136] 4-(2-Oxoethoxy)benzenepropanoic acid (**5**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 6.0 min,
FAB MS: 1669 (M+H),
UV - no maxima.

PREPARATION 34

[0137] 3-(2-Oxoethoxy)benzoic acid (**6**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 5.5 min,
FAB MS: 1641 (M+H),
UV - no maxima.

PREPARATION 35

[0138] 4-(4-Acetylphenoxy)butanoic acid (**7**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 6.4 min,
FAB MS: 1683 (M+H),
UV max at 285 nm (ethanol),
^{1}H-NMR: See Fig. V.

PREPARATION 36

[0139] 4-(4-Acetylphenoxy)butanoic acid (**7**) condensed with calicheamicin gamma dimethyl hydrazide.

HPLC retention time: 6.2 min,
UV max at 285 nm (ethanol).

PREPARATION 37

[0140] 4-(3-Formylphenoxy)butanoic acid (**8**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 6.3 min,
FAB MS: 1669 (M+H).

PREPARATION 38

[0141] 4-(4-Formylphenoxy)butanoic acid (**9**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 6.1 min,
FAB MS: 1669 (M+H),
UV max at 291 nm (ethanol),
^{1}H-NMR: See Fig. VII.

PREPARATION 39

[0142] 4-(4-Acetyl-2-methylphenoxy)butanoic acid (**10**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 6.8 min,
FAB MS: 1697 (M+H).

EXAMPLE 40

[0143] 4-(4-Formyl-2-methoxyphenoxy)butanoic acid (**11**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 5.5 min,
FAB MS: 1699 (M+H),
UV max at 284, 300, and 316 nm (acetonitrile).

EXAMPLE 41

[0144] 4-Formylbenzenepropanoic acid (**12**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 5.6 min,
FAB MS: 1639 (M+H).

EXAMPLE 42

[0145] 4-(2,3-Dimethoxy-5-formylphenoxy)butanoic acid (**13**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 5.8 min,

FAB MS: 1729 (M+H),
UV max at 302 nm (ethanol).

EXAMPLE 43

[0146] 4-(4-Acetyl-2,6-dimethoxyphenoxy)butanoic acid (**14**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 6.0 min,
FAB MS: 1743 (M+H),
UV max at 287 nm (ethanol) .

EXAMPLE 44

[0147] 4-(4-Acetyl-2-methoxyphenoxy)butanoic acid (**15**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 6.1 min,
FAB MS: 1713 (M+H),
UV max at 284 nm (ethanol).

EXAMPLE 45

[0148] 4-[4-(3-Oxobutyl)phenoxy]butanoic acetic acid (**16**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 6.6 min.
FAB MS: 1611 (M+H),
UV - no maxima.

EXAMPLE 46

[0149] 4-(2-Acetyl-5-methoxyphenoxy]butanoic acid (**17**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 6.5 min,
FAB MS: 1713 (M+H),
UV - no maxima.

EXAMPLE 47

[0150] 4-[4-(3-Oxopropyl)phenoxy]butanoic acid (**18**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 9.8 min,
FAB MS: 1697 (M+H),
UV - no maxima.

EXAMPLE 48

[0151] 4-Acetylbenzenebutanoic acid (**19**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 6.4 min,
FAB MS: 1667 (M+H),
UV max at 281 nm (ethanol).

EXAMPLE 49

[0152] 4-[(2-Acetyl-1-naphthalenyl)oxy] butanoic acid (**20**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 7.8 min,
FAB MS: 1733 (M+H),
UV - no maxima.

EXAMPLE 50

[0153] 4-[4-(4-Fluorobenzoyl)phenoxy]butanoic acid (**21**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 8.4 min,
FAB MS: 1763 (M+H),
UV max at 284 nm (ethanol).

EXAMPLE 51

[0154] 4-(4-Acetylphenyl)-1-piperazinepentanoic acid (**22**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 5.0 min,
FAB MS: 1641 (M+H),
UV max at 322 nm (ethanol).

EXAMPLE 52

[0155] 11-(4-Acetylphenoxy)undecanoic acid (**23**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 4.8 min (65% acetonitrile-isocratic),
FAB MS: 1781 (M+H),
UV max at 286 nm (ethanol).

EXAMPLE 53

[0156] 5-[(4-Acetylphenyl)amino]-5-oxopentanoic acid (**24**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 5.2 min,
FAB MS: 1710 (M+H),
UV max at 295 nm (ethanol).

EXAMPLE 54

[0157] 4-(2-Chloro-4-formylphenoxy)butanoic acid (**25**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 6.5 min,
FAB MS: 1704 (M+H),
UV max at 292 nm (ethanol).

EXAMPLE 55

[0158] 5-Acetyl-2-(3-carboxypropoxy)benzoic acid (**26**), methyl ester condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 6.1 min,
FAB MS: 1741 (M+H),
UV max at 285 nm (ethanol).

EXAMPLE 56

[0159] 4-(4-Formyl-2-nitrophenoxy)butanoic acid (**27**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 6.2 min,
FAB MS: 1741 (M+H),
UV max at 294 nm (ethanol).

EXAMPLE 57

[0160] 4-[2-[[(4-Acetylphenyl]amino]methyl]-6-methoxyphenoxy]butanoic acid (**28**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 7.7 min,
FAB MS: 1818 (M+H),
UV max at 323 nm (ethanol).

EXAMPLE 58

[0161] 4-(4-Acetyl-3-fluorophenoxy)butanoic acid (**30**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 6.1 min,
FAB MS: 1701 (M+H),
UV max at 273 nm (ethanol).

EXAMPLE 59

[0162] 4-(2-Acetylphenoxy)butanoic acid (**31**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 6.1 min,
FAB MS: 1683 (M+H),
UV - no maxima.

EXAMPLE 60

[0163] 2-Acetyl-10H-phenothiazine-10-hexanoic acid (**32**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 6.2 min,
FAB MS: 1833 ($M+NH_4$),
UV max at 281, strong shoulder at 356 nm (CH_3CN).

EXAMPLE 61

[0164] 4-Acetylphenylacetic acid (**33**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide.

HPLC retention time: 5.0 min,
FAB MS: 1639 (M+H),
UV max at 281 nm (acetonitrile).

SYNTHESIS OF STRUCTURES C (Scheme 1)

General Procedure

[0165] The carboxylic acid-hydrazones as obtained above are converted to the OSu esters (Z^3=N-succinimidyloxy) by dissolving them in an appropriate solvent such as acetonitrile or acetonitrile containing 10-20% N,N-dimethylforamide or tetrahydrofuran for better solubilization and adding ~2-5 equivalents of N-hydroxysuccinimide and ~2-10 equivalents of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) as the hydrochloride salt. The reaction is allowed to proceed at ambient temperature until complete as measured by HPLC or alternately by TLC, which is usually 1 to 8 hours. The solvents are then removed and the crude product is purified on an appropriate silica gel, such as Biosil-A™, using an appropriate solvent system, such as a gradient of 0 to 20% methanol in either chloroform or ethyl acetate. The products are then sufficiently pure for the conjugation step.

EXAMPLE 62

[0166] 4-Formylphenoxyacetic acid (**1**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 6.5 min.

EXAMPLE 63

[0167] 4-Formylbenzoic acid (**2**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 6.6 min,
FAB MS: 1708 (M+H),
UV max at 310 nm (acetonitrile).

EXAMPLE 64

[0168] 4-Formyl-3-methoxyphenoxyacetic acid (**3**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 7.0 min.
FAB MS: 1768 (M+H),
UV max at 279, 288, and 320 nm (acetonitrile).

EXAMPLE 65

[0169] 6-Formyl-2-naphthoic acid (**4**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 7.4 min,
FAB MS: 1758 (M+H),
UV max at 272 and 323 nm (ethanol).

EXAMPLE 66

[0170] 4-(2-oxoethoxy)benzenepropanoic acid (**5**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 7.1 min.
FAB MS: 1766 (M+H),
UV - no maxima.

EXAMPLE 67

[0171] 3-(2-Oxoethoxy)benzoic acid (**6**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 7.0 min,
UV - no maxima.

EXAMPLE 68A

[0172] 4-(4-Acetylphenoxy)butanoic acid (**7**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 7.5 min,
FAB MS: 1780 (M+H),
UV max at 283 nm (acetonitrile),
^{1}H-NMR: See Fig. VI.

EXAMPLE 68B

[0173] 4-(4-Acetylphenoxy)butanoic acid (**7**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, (1-hydroxy-2,5-dioxo-3-pyrrolidinesulfonic acid, monosodium salt) ester (i.e. ester with "sulfonato-N-hydroxysuccimide").

HPLC retention time: 5.2 min,
UV max at 278 nm (ethanol).

EXAMPLE 69

[0174] 4-(4-Acetylphenoxy)butanoic acid (**7**) condensed with calicheamicin gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 7.6 min,
UV max at 283 nm (acetonitrile).

EXAMPLE 70

[0175] 4-(3-Formylphenoxy)butanoic acid (**8**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 7.4 min,
FAB MS: 1766 (M+H),
UV max at 283 nm (acetonitrile).

EXAMPLE 71

[0176] 4-(4-Formylphenoxy)butanoic acid (**9**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 7.0 min,
FAB MS: 1766 (M+H),
UV max at 289 nm (acetonitrile).

EXAMPLE 72

[0177] 4-(4-Acetyl-2-methylphenoxy)butanoic acid (**10**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 8.2 min,
FAB MS: 1794 (M+H).

EXAMPLE 73

[0178] 4-(4-Formyl-2-methoxyphenoxy)butanoic acid (**11**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 6.6 min,
FAB MS: 1796 (M+H).

EXAMPLE 74

[0179] 4-Formylbenzenepropanoic acid (**12**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 6.7 min,
FAB MS: 1736 6 (M+H).

EXAMPLE 75

[0180] 4-(2,3-Dimethoxy-5-formylphenoxy)butanoic acid (**13**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 6.7 min,
FAB MS: 1826 (M+H),
UV max at 298 nm (ethanol).

EXAMPLE 76

[0181] 4-(4-Acetyl-2, 6-dimethoxyphenoxy)butanoic acid (**14**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 7.7 min,
FAB MS: 1840 (M+H),
UV max at 286 nm (acetonitrile).

EXAMPLE 77

[0182] 4-(4-Acetyl-2-methoxyphenoxy)butanoic acid (**15**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 7.2 min,
FAB MS: 1810 (M+H),
UV max at 284 nm (acetonitrile).

EXAMPLE 78

[0183] 4-[4-(3-Oxobutyl)phenoxy]butanoic acid (**16**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 7.9 min,
FAB MS: 1808 (M+H),
UV - no maxima.

EXAMPLE 79

[0184] 4-(2-Acetyl-5-methoxyphenoxy]butanoic acid (**17**) condensed with Calicheamicin N-acetyl gamma dimethyl

hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 7.4 min,
FAB MS: 1810 (M+H),
UV - no maxima.

EXAMPLE 80

[0185] 4-[4-(3-Oxopropyl)phenoxy]butanoic acid (**18**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 13.1 min,
FAB MS: 1794 (M+H),
UV - no maxima.

EXAMPLE 81

[0186] 4-Acetylbenzenebutanoic acid (**19**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 7.7 min.

EXAMPLE 82

[0187] 4-[(2-Acetyl-1-naphthalenyl)oxy] butanoic acid (**20**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 9.4 min,
FAB MS: 1830 (M+H),
UV - no maxima.

EXAMPLE 83

[0188] 4-[4-(4-Fluorobenzoyl)phenoxy]butanoic acid (**21**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 9.3 min,
FAB MS: 1860 (M+H),
UV max at 284 nm (ethanol).

EXAMPLE 84

[0189] 4-(4-Acetylphenyl)-1-piperazinepentanoic acid (**22**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 6.3 min,
FAB MS: 1863 (M+H),
UV max at 306 nm (1:1 acetonitrile/chloroform).

EXAMPLE 85

[0190] 11-(4-Acetylphenoxy)undecanoic acid (**23**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 15.5 min,
FAB MS: 1878 (M+H),
UV max at 284 nm (ethanol).

EXAMPLE 86

[0191] 5-[(4-Acetylphenyl)amino]-5-oxopentanoic acid (**24**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 6.2 min,
FAB MS: 1807 (M+H),
UV max at 292 nm (acetonitrile).

EXAMPLE 87

[0192] 4-(2-Chloro-4-formylphenoxy)butanoic acid (**25**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 7.5 min,
FAB MS: 1800 (M+H),
UV max at 290 nm (acetonitrile).

EXAMPLE 88

[0193] 5-Acetyl-2-(3-carboxypropoxy)benzoic acid (**26**), methyl ester condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 7.2 min,
FAB MS: 1838 (M+H),
UV max at 284 nm (acetonitrile).

EXAMPLE 89

[0194] 4-{4-Formyl-2-nitrophenoxy)butanoic acid (**27**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 7.1 min,
FAB MS: 1811 (M+H),
UV max at 293 nm (ethanol).

EXAMPLE 90

[0195] 4-[2-[[(4-Acetylphenyl)amino]methyl]-6-methoxyphenoxy]butanoic acid (**28**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 9.2 min,
FAB MS: 1916 (M+H),
UV max at 309 nm (acetonitrile).

EXAMPLE 91

[0196] 4-(4-Acetyl-3-fluorophenoxy)butanoic acid (**30**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 8.2 min,
FAB MS: 1798 (M+H),
UV max at 270 nm (ethanol).

EXAMPLE 92

[0197] 4-(2-Acetylphenoxy)butanoic acid (**31**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 8.1 min,
FAB MS: 1780 (M+H),
UV - no maxima.

EXAMPLE 93

[0198] 2-Acetyl-10H-phenothiazine-10-hexanoic acid (**32**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 8.3 min,
FAB MS: 1930 ($M+NH_4$),
UV max at 281 nm (acetonitrile).

EXAMPLE 94

[0199] 4-Acetylphenylacetic acid (**33**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide, N-hydroxysuccimide ester.

HPLC retention time: 7.2 min,
FAB MS: 1736 (M+H),
UV max at 280 nm (acetonitrile).

SYNTHESIS OF STRUCTURES D (Method A-Scheme 1)

General Procedure

[0200] The activated ester from above is dissolved in an, appropriate organic solvent, such as dimethylformamide, and added to a solution of antibody at ~1-15 mg/mL in an appropriate buffer, such as pH 7.4 phosphate (50 mM, 100 mM salt) such that the concentration of organic co-solvent is ~10-30% and ~2-10 equivalents of active ester are used per mole of antibody. The conjugation reaction is allowed to proceed at ambient temperature for ~4-24 hours. The solution is concentrated by use of a semipermeable membrane, if necessary, and purified by standard size-exclusion chromatography, such as with Sephacryl™ S-200 gel. The monomer fractions are pooled and the loading of drug on the antibody is estimated by UV-VIS absorbance at 280 nm for antibody and 333 nm or other appropriate wavelength for the calicheamicin hydrazones.

SYNTHESIS OF STRUCTURES E (Scheme 2)

General Procedure

[0201] The carboxylic acids of the spacers are activated as the OSu esters (Z^3=N-succinimidyloxy) by dissolving them in an appropriate solvent such as tetrahydrofuran containing 10-20% dimethylformamide and adding -2-3 equivalents of N-hydroxysuccinimide and ~2-5 equivalents of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) as the hydrochloride salt. The reaction is allowed to proceed at ambient temperature until complete as assessed by TLC, which is usually 1 to 8 hours. The solvents are then removed and the crude product is purified on an appropriate silica gel, such as Biosil-A™, using an appropriate solvent system, such as a gradient of 0 to 5% methanol in chloroform. The products are generally purified further by recrystallization from a mixture of ethyl acetate-hexanes or other appropriate solvents.

[0202] The following preparations were made by the above procedure:

(SuOH = N-hydroxysuccinimide)

PREPARATION 95

4-Formylphenoxy acetic acid (**1**), N-hydroxysuccinimide ester.

[0203] CI MS: 278 (MH^+), NMR ($CDCl_3$+D_6-DMSO): 9.9 ppm (1H, s, CH=O), 7.9 and 7.1 (2H each, d, ArH), 5.2 (2H, s, OCH_2), 2.9 (4H, s, CH_2CH_2).

PREPARATION 96

4-Formyl-3-methoxyphenoxy acetic acid (**3**), N-hydroxysuccinimide ester.

[0204] CI MS: 308 (MH^+), NMR ($CDCl_3$): 10.3 ppm (1H, s, CH=O), 7.8 (1H, d, ArH), 6.6 (1H, dt, ArH), 6.55 (1H, d, ArH), 5.1 (2H, s, OCH_2), 3.95, (3H, s, OCH_3), 2.9 (4H, s, CH_2CH_2).

PREPARATION 97

4-(4-Acetylphenoxy)butanoic acid (**7**), N-hydroxysuccinimide ester.

[0205] CI MS: 320 (MH^+), NMR ($CDCl_3$): 7.9 and 7.0 (2H each, d, ArH), 4.2 (2H, s, OCH_2), 2.9 (6H, m, CH_2CH_2 + $O=CCH_2$), 2.6 (3H, s, $O=CCH_3$), 2.3 (2H, m, CH_2).

SYNTHESIS OF STRUCTURES F (Scheme 2)

General Procedure

[0206] The activated ester from above is dissolved in an appropriate organic solvent, such as N,N-dimethylformamide, and added to a solution of antibody at ~1-15 mg/mL in an appropriate buffer, such as pH 7.4 phosphate (50 mM, 100 mM salt) such that the concentration of organic co-solvent is ~10-25% and ~2-20 equivalents of active ester are used per mole of antibody. The conjugation reaction is allowed to proceed at ambient temperature for ~4-24 hours. The buffer is exchanged and the organic co-solvents and by-products are removed by use of a desalting column such as a PD-10 using pH 5.5 acetate buffer (25 mM acetate, 100 mM NaCl). The solution is concentrated by use of a semipermeable membrane, if necessary, and the product is used without further purification for the following step. The number of carbonyl groups incorporated per antibody is usually about half the number of equivalents of OSu ester used and can be further quantified by use of *p*-nitrophenyl hydrazine or other comparable method, if desired.

SYNTHESIS OF STRUCTURES D (Method B-Scheme 2)

General Procedure

[0207] The drug hydrazide derivative is dissolved in an appropriate organic solvent, such as N,N-dimethylformamide, and added to a solution of antibody-linker conjugate (structure F) from the previous step at ~1-15 mg/mL in an appropriate buffer, such as pH acetate (25 mM, 100 mM salt) such that the concentration of organic co-solvent is ~10-15% and ~2-15 equivalents of hydrazide are used per mole of antibody. The conjugation reaction is allowed to proceed at ambient temperature for ~4-24 hours. The buffer is exchanged and the organic co-solvents and by-products are removed by use of a desalting column such as a PD-10 using pH 7.4 buffer (50 mM phosphate, 100 mM NaCl). The solution is concentrated by use of a semipermeable membrane, if necessary, and purified by standard size-exclusion chromatography, such as with Sephacryl™ S-200 gel. The monomer fractions are pooled and the loading of drug on the antibody is estimated by UV-VIS absorbence at 280 nm for antibody and 333 nm or other appropriate wavelength for the calicheamicin hydrazones.

EXAMPLE 98 (METHOD A AND B)

[0208] conjugate of 4-formylphenoxyacetic acid (**1**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 1.0 M/M,	Rel. Affinity: 0.65,
In vitro IC_{50}: 0.23 ng/mL,	Spec. Index: 1,600,
In vivo: 29% T/C (2 μg x 3 doses -- 5/5 alive-28d),	
Ex vivo: 95% inhibition.	

EXAMPLE 99 (METHOD A AND B)

[0209] Conjugate of 4-formylphenoxyacetic acid (**1**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-P67.6.

Loading: 1.5 M/M, *In vitro* IC_{50}: 0.068 ng/mL, *Ex vivo*: 90% inhibition.	Rel. Affinity: 0.77, Spec. Index: 3,600,

EXAMPLE 100 (METHOD A)

[0210] Conjugate of 4-formylphenoxyacetic acid (**1**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-CT-M-01.

Loading: 2.0. M/M, *In vitro* IC_{50}: 1.5 ng/mL,	Rel. Affinity: 0.84, Spec. Index: 59.

EXAMPLE 101 (METHOD A)

[0211] Conjugate of 4-formylbenzoic acid (**2**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 4.8 M/M, *In vitro* IC_{50}: 4.8 ng/mL, *Ex vivo*: 63% inhibition.	Rel. Affinity: 0.99, Spec. Index: >125,

EXAMPLE 102 (METHOD A)

[0212] Conjugate of 4-formylbenzoic acid (**2**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-P67.6.

Loading: 4.0 M/M, *In vitro* IC_{50}: 4.0 ng/mL,	Rel. Affinity: 1.05, Spec. Index: >125.

EXAMPLE 103 (METHOD A)

[0213] Conjugate of 4-formylbenzoic acid (**2**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-CT-M-01.

Loading: 2.3 M/M, *In vitro* IC_{50}: 5.6 ng/mL,	Rel. Affinity: 0.90, Spec. Index: 32.

EXAMPLE 104 (METHOD A AND B)

[0214] Conjugate of 4-formyl-3-methoxyphenoxyacetic acid (**3**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 0.8 M/M, *In vitro* IC_{50}: 0.30 ng/mL,	Rel. Affinity: 0.81, Spec. Index: 375.

EXAMPLE 105 (METHOD A AND B)

[0215] Conjugate of 4-formyl-3-methoxyphenoxyacetic acid (**3**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-P67.6.

Loading: 0.9 M/M, *In vitro* IC_{50}: 0.12 ng/mL, *Ex vivo:* 90% inhibition.	Rel. Affinity: 0.76, Spec. Index: 1,200,

EXAMPLE 106 (METHOD A)

[0216] Conjugate of 4-formyl-3-methoxyphenoxyacetic acid (**3**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-CT-M-01.

Loading: 2.1 M/M, *In vitro* IC_{50}: 5.6 ng/mL,	Rel. Affinity: 0.88, Spec. Index: 12.

EXAMPLE 107 (METHOD A)

[0217] Conjugate of 6-formyl-2-naphthoic acid (**4**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-P67.6.

Loading: 2.0 M/M, *In vitro* IC_{50}: 0.047 ng/mL,	Rel. Affinity: 0.73, Spec. Index: 675.

EXAMPLE 108 (METHOD A)

[0218] Conjugate of 4-(2-oxoethoxy)benzenepropanoic acid (**5**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 1.1 M/M, *In vitro* IC_{50}: 2.22 ng/mL,	Rel. Affinity: 1.09, Spec. Index: 125.

EXAMPLE 109 (METHOD A)

[0219] Conjugate of 4-(2-oxoethoxy)benzenepropanoic acid (**5**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-P67.6.

Loading: 0.6 M/M, *In vitro* IC_{50}: 0.45 ng/mL, *Ex vivo:* 71% inhibition.	Rel. Affinity: 1.11, Spec. Index: 200,

EXAMPLE 110 (METHOD A)

[0220] Conjugate of 3-(2-oxoethoxy)benzoic acid (**6**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 1.3 M/M, *In vitro* IC_{50}: 0.69 ng/mL,	Rel. Affinity: 1.19, Spec. Index: 100.

EXAMPLE 111 (MRTHOD A)

[0221] Conjugate of 3-(2-oxoethoxy)benzoic acid (**6**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-P67.6.

Loading: 0.8 M/M, *In vitro* IC_{50}: 0.32 ng/mL,	Rel. Affinity: 1.91, Spec. Index: 175.

EXAMPLE 112 (METHOD A)

[0222] Conjugate of 4-(4-acetylphenoxy)butanoic acid (**7**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 2.7 M/M,	Rel. Affinity: 0.75,

(continued)

In vitro IC_{50}: 0.098 ng/mL,	Spec. Index: 6,400,
In vivo: 0% T/C (1 μg x 3 doses, 5/5 alive-28d),	
0% T/C (3 μg x 3 doses, 5/5 alive-28d),	
0% T/C (6 μg x 3 doses, 5/5 alive-28d),	
Ex vivo: 96% inhibition.	

EXAMPLE 113 (METHOD A)

[0223] Conjugate of 4-(4-acetylphenoxy)butanoic acid (**7**) condensed with calicheamicin gamma dimethyl hydrazide and h-P67.6.

Loading: 3.2 M/M,	Rel. Affinity: 0.78,
In vitro IC_{50}: 0.001 ng/mL,	Spec. Index: 10,000

EXAMPLE 114 (METHOD A OR B)

[0224] Conjugate of 4-(4-acetylphenoxy)butanoic acid (**7**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-P67.6.

Loading: 1.7 M/M,	Rel. Affinity: 0.96,
In vitro IC_{50}: 0.017 ng/mL,	Spec. Index: 29,500,
In vivo: 22% T/C (0.5 μg x 3 doses, 5/5 alive-28d),	
0% T/C (1 μg x 3 doses, 5/5 alive-28d),	
1% T/C (3 μg x 3 doses, 5/5 alive-28d),	
0% TIC (6 μg x 3 doses, 2/5 alive-28d),	
Ex vivo: 98% inhibition.	

EXAMPLE 115 (METHOD A)

[0225] Conjugate of 4-(4-acetylphenoxy)butanoic acid (**7**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-CT-M-01.

Loading: 3.4 M/M,	Rel. Affinity: not determined,
In vitro IC_{50}: 0.048 ng/mL,	Spec. Index: 6,200.

EXAMPLE 116 (METHOD A)

[0226] Conjugate of 4-(4-acetylphenoxy)butanoic acid (**7**) condensed with calicheamicin N-acetyl gamma dimethyl hydrazide and m-A33.

Loading: 1.1 M/M,	Rel. Affinity: 0.68,
In vitro IC_{50}: 3.32 ng/mL,	Spec. Index: 0.72.
In vivo: 4% T/C (3 μg x 3 doses, 5/5 alive-28d),	
5% T/C (6 μg x 3 doses, 5/5 alive-28d).	

EXAMPLE 117 (METHOD A)

[0227] Conjugate of 4-(4-acetylphenoxy)butanoic acid (**7**) condensed with calicheamicin N-acetyl gamma dimethyl hydrazide and h-A33.

Loading: 1.8 M/M. *In vitro* IC_{50}: 4.03 ng/mL,	Rel. Affinity: 1.13, Spec. Index: 0.96.

EXAMPLE 118 (METHOD A)

[0228] Conjugate of 4-(4-acetylphenoxy)butanoic acid (**7**) condensed with calicheamicin gamma dimethyl hydrazide and h-A33.

Loading: 2.8 M/M, *In vitro* IC_{50}: 3.55 ng/mL,	Rel. Affinity: 0.91, Spec. Index: 2.6.

EXAMPLE 119 (METHOD A)

[0229] Conjugate of 4-(4-acetylphenoxy)butanoic acid (**7**) condensed with calicheamicin N-acetyl gamma dimethyl hydrazide and anti-Tac.

Loading: 2.1 M/M, *In vitro* IC_{50}: 0.004 ng/mL, *Ex vivo*: IC_{50}:1.0 ng/mL,	Rel. Affinity: not determined, Spec. Index: 250, Spec. Index: 100.

EXAMPLE 120 (METHOD A)

[0230] Conjugate of 4-(3-formylphenoxy)butanoic acid (**8**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-P67.6.

Loading: 1.7 M/M, *In vitro* IC_{50}: 0.38 ng/mL,	Rel. Affinity: 1.00, Spec. Index: 1,700.

EXAMPLE 121 (METHOD A)

[0231] Conjugate of 4-(4-formylphenoxy)butanoic acid (**9**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 2.8 M/M, *In vitro* IC_{50}; 0.52 ng/mL,	Rel. Affinity: 0.56, Spec. Index: 2,900,
In vivo: 12% T/C (1 μg x 3 doses, 5/5 alive-28d), 9% T/C (3 μg x 3 doses, 5/5 alive-28d), 3% T/C (6 μg x 3 doses, 4/5 alive-28d),	
Ex vivo: 98% inhibition.	

EXAMPLE 122 (METHOD A)

[0232] Conjugate of 4-(4-formylphenoxy)butanoic acid (**9**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-P67.6.

Loading: 1.8 M/M, *In vitro* IC_{50}: 0.087 ng/mL,	Rel. Affinity: 0.70, Spec. Index: 11,000,
In vivo: 17% T/C (0.5 μg x 3 doses, 5/5 alive-28d), 23% T/C (1 μg x 3 doses, 5/5 alive-28d), 9% T/C (3 μg x 3 doses, 5/5 alive-28d), 0% T/C (6 μg x 3 doses, 5/5 alive-28d).	

EXAMPLE 123 (METHOD A)

[0233] Conjugate of 4-(4-acetyl-2-methylphenoxy)butanoic acid (**10**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 3.5 M/M, *In vitro* IC_{50}: 0.45 ng/mL,	Rel. Affinity: 1.16, Spec. Index: 2,900.

EXAMPLE 124 (METHOD A)

[0234] Conjugate of 4-(4-acetyl-2-methylphenoxy)butanoic acid (**10**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-P67.6.

Loading: 1.6 M/M, *In vitro* IC_{50}: 0.041 ng/mL,	Rel. Affinity: 1.07. Spec. Index: 5,100.

EXAMPLE 125 (METHOD A)

[0235] Conjugate of 4-(4-formyl-2-methoxyphenoxy)butanoic acid (**11**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 2.6 M/M, *In vitro* IC_{50}: 3.8 ng/mL,	Rel. Affinity: 0.73, Spec. Index: 575.

EXAMPLE 126 (METHOD A)

[0236] Conjugate of 4-(4-formyl-2-methoxyphenoxy)butanoic acid (**11**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-P67.6.

Loading: 1.9 M/M, *In vitro* IC_{50}: 0.13 ng/mL,	Rel. Affinity: 0.22, Spec. Index: 1,800.

EXAMPLE 127 (METHOD A)

[0237] Conjugate of 4-formylbenzenepropanoic acid (**12**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 2.5 M/M, *In vitro* IC_{50}: 1.0 ng/mL,	Rel. Affinity: 0.73, Spec. Index: 950.

EXAMPLE 128 (METHOD A)

[0238] Conjugate of 4-formylbenzenepropanoic acid (**12**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-P67.6.

Loading: 1.6 M/M, *In vitro* IC_{50}: 0.12 ng/mL,	Rel. Affinity: 0.73, Spec. Index: 2,000.

EXAMPLE 129 (METHOD A)

[0239] Conjugate of 4-(2,3-dimethoxy-5-formylphenoxy)butanoic acid (**13**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 1.0 M/M, *In vitro* IC_{50}: 1.1 ng/mL,	Rel. Affinity: 1.16, Spec. Index: >375.

EXAMPLE 130 (METHOD A)

[0240] Conjugate of 4-(2,3-Dimethoxy-5-formylphenoxy)butanoic acid (**13**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-P67.6.

Loading: 1.8 M/M,	Rel. Affinity: 1.08,
In vitro IC_{50}: 0.062 ng/mL,	Spec. Index: >9,800.

EXAMPLE 131 (METHOD A)

[0241] Conjugate of 4-(4-acetyl-2,6-dimethoxyphenoxy)butanoic acid (**14**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 2.6 M/M,	Rel. Affinity: 1.07,
In vitro IC_{50}: 0.24 ng/mL,	Spec. Index: >1,700.

EXAMPLE 132 (METHOD A)

[0242] Conjugate of 4- (4-acetyl-2, 6-dimethoxyphenoxy)butanoic acid (**14**) condensed, with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-P67.6.

Loading: 1.7 M/M,	Rel. Affinity: 1.18,
In vitro IC_{50}: 0.015 ng/mL,	Spec. Index: >40,500.

EXAMPLE 133 (METHOD A)

[0243] Conjugate of 4-(4-acetyl-2-methoxyphenoxy)butanoic acid (**15**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 2.3 M/M,	Rel. Affinity: 0.78,
In vitro IC_{50}: 0.23 ng/mL,	Spec. Index: 875.

EXAMPLE 134 (METHOD A)

[0244] Conjugate of 4-(4-acetyl-2-methoxyphenoxy)butanoic acid (**15**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-P67.6.

Loading: 1.7 M/M,	Rel. Affinity: 0.80,
In vitro IC_{50}: 0.029 ng/mL,	Spec. Index: 13,500.

EXAMPLE 135 (METHOD A)

[0245] Conjugate of 4-[4-(3-oxobutyl)phenoxy]butanoic acid (**16**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-P67. 6.

Loading: 0.5 M/M,	Rel. Affinity: not determined,
In vitro IC_{50}: 9 ng/mL,	Spec. Index: 2.

EXAMPLE 136 (METHOD A)

[0246] Conjugate of 4-(2-acetyl-5-methoxyphenoxy)butanoic acid (**17**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 2.3 M/M,	Rel. Affinity: 0.98,
In vitro IC_{50}: 0.088 ng/mL,	Spec. Index: 1,100.

EXAMPLE 137 (METHOD A)

[0247] Conjugate of 4-(2-acetyl-5-methoxyphenoxy]butanoic acid (**17**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-P67.6.

Loading: 1.6 M/M, *In vitro* IC_{50}: 0.0098 ng/mL,	Rel. Affinity: 1.20, Spec. Index: 21,500.

EXAMPLE 138 (METHOD A)

[0248] Conjugate of 4-[4-(3-oxopropyl)phenoxy]butanoic acid (**18**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 1.0 M/M, *In vitro* IC_{50}: 1.1 ng/mL,	Rel. Affinity: 0.80, Spec. Index: 80.

EXAMPLE 139 (METHOD A)

[0249] Conjugate of 4-[4- (3-oxopropyl)phenoxy]butanoic acid (**18**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-P67.6.

Loading: 0.6 M/M, *In vitro* IC_{50}: 0.62 ng/mL,	Rel. Affinity: 1.21, Spec. Index: 90.

EXAMPLE 140 (METHOD A)

[0250] Conjugate of 4-acetylbenzenebutanoic acid (**19**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 2.6 M/M, *In vitro* IC_{50}: 0.012 ng/mL,	Rel. Affinity: 0.50, Spec. Index: 2,600.
In vivo: 23% T/C (0.5 μg x 3 doses, 5/5 alive-28d), 10% T/C (1 μg x 3 doses, 5/5 alive-28d), 4% T/C (3 μg x 3 doses, 4/5 alive-28d), 0% T/C (6 μg x 3 doses, 2/5 alive-28d),	
Ex vivo: 99% inhibition.	

EXAMPLE 141 (METHOD A)

[0251] Conjugate of 4-acetylbenzenebutanoic acid (**19**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-P67.6.

Loading: 2.2 M/M, *In vitro* IC_{50}: 0.0082 ng/mL,	Rel. Affinity: 0.42, Spec. Index: 31,500,
In vivo: 21% T/C (0.5 μg x 3 doses, 5/5 alive-28d), 25% T/C (1 μg x 3 doses, 5/5 alive-28d) 0% T/C (3 μg x 3 doses, 4/5 alive-28d), 0% T/C (6 μg x 3 doses, 1/5 alive-28d),	
Ex vivo: 99% inhibition.	

EXAMPLE 142 (METHOD A)

[0252] Conjugate of 4- [(2-acetyl-1-naphthalenyl)oxy] butanoic acid (**20**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 2.5 M/M, *In vitro* IC_{50}: 0.061 ng/mL,	Rel. Affinity: 0.50, Spec. Index: 5,000,
In vivo: 36% T/C (1 μg x 3 doses, 5/5 alive-28d), 22% T/C (3 μg x 3 doses, 5/5 alive-28d), 11% T/C (6 μg x 3 doses, 4/5 alive-28d), *Ex vivo*: 76% inhibition.	

EXAMPLE 143 (METHOD A)

[0253] Conjugate of 4- [(2-acetyl-1-naphthalenyl)oxy] butanoic acid (**20**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-P67.6.

Loading: 1.8 M/M, *In vitro* IC_{50}: 0.0067	Rel. Affinity: 0.66, Spec. Index: 105,000.

EXAMPLE 144 (METHOD A)

[0254] Conjugate of 4-[4- (4-fluorobenzoyl)phenoxy]butanoic acid (**21**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 2.5 M/M, *In vitro* IC_{50}: 99 ng/mL,	Rel. Affinity: 0.67, Spec. Index: 3.

EXAMPLE 145 (METHOD A)

[0255] Conjugate of 4-[4-(4-fluorobenzoyl)phenoxy]butanoic acid (**21**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 1.8 M/M, *In vitro* IC_{50}: 63 ng/mL,	Rel. Affinity: 0.76, Spec. Index: 9.

EXAMPLE 146 (METHOD A)

[0256] Conjugate of 4-(4-acetylphenyl)-1-piperazinepentanoic acid (**22**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 0.1 M/M, *In vitro* IC_{50}: 12 ng/mL,	Rel. Affinity: 0.98, Spec. Index: 2.

EXAMPLE 147 (METHOD A)

[0257] Conjugate of 11-(4-acetylphenoxy)undecanoic acid (**23**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 0.5 M/M, *In vitro* IC_{50}: 0.43 ng/mL,	Rel. Affinity: 0.80, Spec. Index: 175.

EXAMPLE 148 (METHOD A)

[0258] Conjugate of 11-(4-acetylphenoxy)undecanoic acid (**23**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-P67.6.

Loading: 0.4 M/M, *In vitro* IC_{50}: 0.47 ng/mL,	Rel. Affinity: 1.16, Spec. Index: 125.

EXAMPLE 149 (METHOD A)

[0259] Conjugate of 5-[(4-acetylphenyl)amino]-5-oxopentanoic acid (**24**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and m-P67.6.

Loading: 2.0 M/M, *In vitro* IC_{50}: <0.005 ng/mL,	Rel. Affinity: 0.73, Spec. Index: >1,200.

EXAMPLE 150 (METHOD A)

[0260] Conjugate of 4-(2-chloro-4-formylphenoxy)butanoic acid (**25**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 2.0 M/M, *In vitro* IC_{50}: 0.0071 ng/mL,	Rel. Affinity: 0.31, Spec. Index: 1,500.

EXAMPLE 151 (METHOD A)

[0261] Conjugate of 5-acetyl-2-(3-carboxypropoxy)benzoic acid (**26**), methyl ester condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 2.0 M/M, *In vitro* IC_{50}: <0.005 ng/mL,	Rel. Affinity: 0.79, Spec. Index: >9,600.

EXAMPLE 152 (METHOD A)

[0262] Conjugate of 4-(4-formyl-2-nitrophenoxy)butanoic acid (**27**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 1.5 M/M, *In vitro* IC_{50}: 0.023 ng/mL,	Rel. Affinity: 1.3, Spec. Index: >4,500.

EXAMPLE 153 (METHOD A)

[0263] Conjugate of 4-[2-[[(4-acetylphenyl)amino]methyl]-6-methoxyphenoxy]butanoic acid (**28**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 2.0 M/M, *In vitro* IC_{50}: <0.005 ng/mL,	Rel. Affinity: 0.85, Spec. Index: >5,000.

EXAMPLE 154 (METHOD A)

[0264] Conjugate of 4-(4-acetyl-3-fluorophenoxy)butanoic acid (**30**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 1.5 M/M, *In vitro* IC_{50}: 0.005 ng/mL,	Rel. Affinity: 1.01, Spec. Index: 4,800.

EXAMPLE 155 (METHOD A)

[0265] Conjugate of 4-(2-Acetylphenoxy)butanbic acid (**31**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 1.5 M/M, *In vitro* IC_{50}: <0.005 ng/mL,	Rel. Affinity: 0.95, Spec. Index: >7,000.

EXAMPLE 156 (METHOD A)

[0266] Conjugate of 2-acetyl-10H-phenothiazine-10-hexanoic acid (**32**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 1.5 M/M, *In vitro* IC_{50}: 0.021 ng/mL,	Rel. Affinity: 1.25, Spec. Index: 2,300.

EXAMPLE 157 (METHOD A)

[0267] Conjugate of 4-acetylphenylacetic acid (**33**) condensed with Calicheamicin N-acetyl gamma dimethyl hydrazide and h-P67.6.

Loading: 1.4 M/M, *In vitro* IC50: <0.005 ng/mL,	Rel. Affinity: 0.91, Spec. Index: 4,700.

[0268] The described conjugates are useful for inhibiting the growth of unwanted cells which is an important part of the invention. Accordingly, the invention also includes pharmaceutical compositions, most preferably a parenteral composition suitable for injection into the body of a warm-blooded mammal. Such compositions are formulated by methods which are commonly used in pharmaceutical chemistry. The conjugates are acceptably soluble in physiologically acceptable fluids, such as physiological saline solutions and other aqueous solutions which can safely be administered parenterally.

[0269] Products for parenteral administration are often formulated and distributed in solid, preferably freeze dried form, for reconstitution immediately before use. Such formulations are useful compositions of the present invention. Their preparation is well understood by pharmaceutical chemists; in general, they comprise mixtures of inorganic salts, to confer isotonicity, and dispersing agents, such as sucrose, to allow the dried preparation to dissolve quickly upon reconstitution. Such formulations are reconstituted with highly purified water or physiologically acceptable buffers to a known concentration, based on the drug.

[0270] The invention also provides a freeze-dried pharmaceutical composition for inhibiting the growth of cells which is obtained by freeze-drying an approximately 1 mg/ml solution of the conjugate dissolved in about 5 mM sodium phosphate buffer at a pH of about 7.4 containing about 100 mM sodium chloride and about 100 mM sucrose. Preferably, for the conjugate, which has the formula $Z^3[CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=Z^2]m$, Z^3 is antibody h-CT-M-01 or h-p67.6; Alk^1 is C_4 alkylene; Sp^1 is -O-; Ar is 1,4-phenylene; Alk^2 and Sp^2 are together a bond; Z^1 is C_1 alkyl; and Z^2 is calicheamicin N-acetyl gamma dimethyl hydrazide.

[0271] The optimum dosage and administration schedule of conjugates of the invention must be determined by the treating physician, in light of the patient's condition.

[0272] It is customary, of course, to administer cytotoxic drugs in the form of divided doses, with intervals of days or weeks between each series of doses. The conjugates are effective over a wide dosage range, and dosages per week will usually fall within the range from about 1 to about 10,000 μg/m2 of drug, more preferably in the range from about 10 to about 200 $\mu g/m^2$.

Claims

1. A cytotoxic drug conjugate of formula:

$$Z^3(CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=Z^2)m$$

wherein

Z^3 is a protein selected from mono- and polyclonal antibodies, their antigen-recognizing fragments, and their chemically or genetically manipulated counterparts, and growth factors and their chemically or genetically manipulated counterparts, wherein a covalent bond to the protein is an amide formed from reaction with "m" lysine side chains, or a steroid, wherein the covalent bond to the steroid is an amide or an ester;

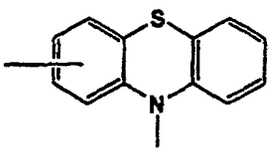
Alk^1 and Alk^2 are independently a bond or branched or unbranched (C_1-C_{10}) alkylene chain;

Sp^1 is a bond, -S-, -O-, -CONH-, -NHCO-, -NR'-, $-N(CH_2CH_2)_2N-$, or -X-Ar'-Y-$(CH_2)_n$-Z wherein X, Y, and Z are independently a bond, -NR'-, -S-, or -O-, with the proviso that when n = 0, then at least one of Y and Z must be a bond and Ar' is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$, with the proviso that when Alk^1 is a bond, Sp^1 is a bond;

n is an integer from 0 to 5;

R' is a branched or unbranched (C_1-C_5) chain optionally substituted by one or two groups of-OH, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, (C_1-C_3) dialkylamino, or (C_1-C_3) trialkylammonium $-A^-$ where A^- is a pharmaceutically acceptable anion completing a salt;

Ar is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, or COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ wherein n and R' are as defined above or a 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6-, or 2,7-naphthylidene or

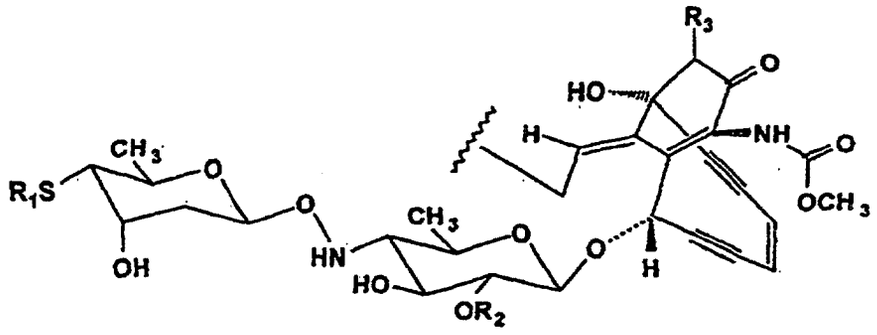


each naphthylidene or phenothiazine optionally substituted with one, two, three, or four groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, or COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ wherein n and R' are as defined above, with the proviso that when Ar is naphthylidene, Z^1 is not hydrogen and with the proviso that when Ar is phenothiazine, Sp^1 is a bond only connected to nitrogen;

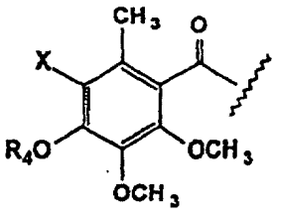
Sp^2 is a bond, -S-, or -O-, with the proviso that when Alk^2 is a bond, Sp^2 is a bond;

Z^1 is H, (C_1-C_5) alkyl, or phenyl optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ wherein n and R' are as defined above;

Z^2 is Q-Sp-S-S-W. wherein W is

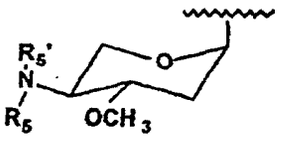


R_1 is



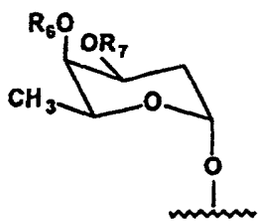
or CH_3;

R_2 is



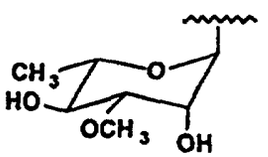
or H;

R_3 is



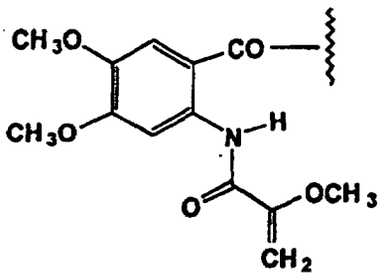
or H;

R_4 is



or H;

R_6 or R_7 is H or



;

R_5 is $-CH_3$, $-C_2H_5$, or $-CH(CH_3)_2$; X is an iodine or bromine atom; R_5' is a hydrogen or the group RCO, wherein R is hydrogen, branched or unbranched (C_1 - C_{10}) alkyl or (C_1 - C_{10}) alkylene group, a (C_6-C_{11}) aryl group, a (C_6-C_{11}) aryl-alkyl (C_1 - C_5) group, or a heteroaryl or heteroaryl-alkyl (C_1 - C_5) group wherein heteroaryl is 2- or 3-furyl, 2- or 3-thienyl, 2-or 3-(N-methylpyrrolyl), 2-, 3-, or 4-pyridyl, 2-, 4-, or 5-(N-methylimidazolyl), 2-, 4-, or 5-oxazolyl, 2-, 3-, 5-, or 6-pyrimidinyl, 2-, 3-, 4-, 5-, 6-, 7-, or 8-quinolyl, or 1-, 3-, 4-, 5-, 6-, 7-, or 8-isoquinolyl, all aryl and heteroaryl optionally substituted by one or more hydroxy, amino, carboxy, halo, nitro, lower (C_1 - C_3) alkoxy, or lower (C_1 - C_5) thioalkoxy groups;

Sp is a straight or branched-chain divalent or trivalent (C_1 - C_{18}) radical, divalent or trivalent aryl or heteroaryl radical, divalent or trivalent (C_3 - C_{18}) cycloalkyl or heterocycloalkyl radical, divalent or trivalent aryl- or heteroaryl-alkyl (C_1 - C_{18}) radical, divalent or trivalent cycloalkyl- or heterocycloalkyl-alkyl (C_1 - C_{18}) radical or divalent or trivalent (C_2 - C_{18}) unsaturated alkyl radical, wherein heteroaryl is furyl, thienyl, N-methylpyrrolyl, pyridinyl, N-methylimidazolyl, oxazolyl, pyrimidinyl, quinolyl, isoquinolyl, N-methylcarbazoyl, aminocoumarinyl, or phenazinyl and wherein when Sp is a trivalent radical, Sp can be additionally substituted by lower (C_1 - C_5) dialkylamino, lower (C_1 - C_5) alkoxy, hydroxy, or lower (C_1 - C_5) alkylthio groups; and

Q is =NHNCO-, =NHNCS-, =NHNCONH-, =NHNCSNH-, or =NHO-

m is from about 0.1 to 15.

2. A cytotoxic drug conjugate according to claim 1, wherein Alk^2 is a branched or unbranched (C_1-C_{10}) alkylene chain and Z^1 is phenyl optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ wherein n and R' are as defined in claim 1.

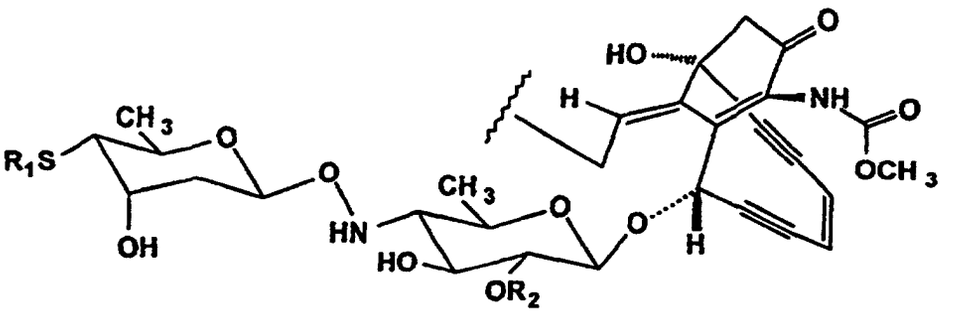
3. A cytotoxic drug conjugate according to claim 1, wherein Alk^2 and Sp^2 are together a bond and Z^1 is H or (C_1-C_5) alkyl.

4. A cytotoxic drug conjugate according to claim 1, wherein Sp^1 is a bond, -S-, -O-, -CONH-, -NHCO-, or -NR' wherein R' is defined in claim 1, with the proviso that when Alk^1 is a bond, Sp^1 is a bond.

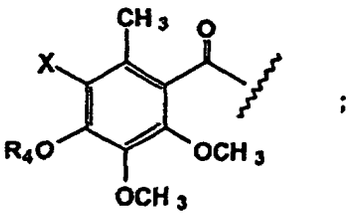
5. A cytotoxic drug conjugate according to claim 4 wherein Ar is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, or COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ wherein n and R' are as defined in claim 1 or Ar is a 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6-, or 2,7-naphthylidene each optionally substituted with one, two, three, or four groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, or COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ wherein n and R' are as defined in claim 1.

6. A cytotoxic drug conjugate according to claim 5 wherein Z^3 is a protein selected from mono- and polyclonal antibodies, their antigen-recognizing fragments, and their chemically or genetically manipulated counterparts and growth factors and their chemically or genetically manipulated counterparts, wherein a covalent bond to the protein is an amide formed from reaction with "m" lysine side chains.

7. A cytotoxic drug conjugate according to claim 6 wherein Z^2 is Q-Sp-S-S-W, wherein W is

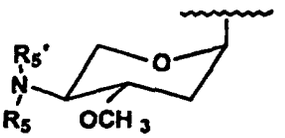


R_1 is



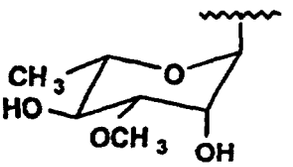
;

R_2 is



or H;

R_4 is



or H;

R_5, X, R_5', R, and Sp are as defined in claim 1; and Q is =NHNCO-.

8. A cytotoxic drug conjugate according to claim 7 wherein Ar is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, or COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ wherein n and R' are as defined in claim 1, Alk^2 and Sp^2 are together a bond, and Z^1 is H or (C_1-C_5) alkyl.

9. A cytotoxic drug conjugate according to claim 8, wherein Sp^1 is -O- or a bond, Alk^1 is C_1 to C_6 alkylene, Ar is 1,3- or 1,4-phenylene optionally substituted with one or two groups of (C_1-C_3) alkyl, (C_1-C_3) alkoxy, halogen,_nitro,

COOR', or CONHR' wherein R' is as defined in claim 1, and Z^1 is (C_1-C_3) alkyl.

10. A cytotoxic drug conjugate according to claim 9, wherein Z^3 is a monoclonal antibody which recognizes the CD33 antigen andZ^2 is calicheamicin gamma dimethyl hydrazide or calicheamicin N-acetyl gamma dimethyl hydrazide.

11. A cytotoxic drug conjugate according to claim 9, wherein Z^3 is a monoclonal antibody which recognizes the polyepithelial mucin antigen and Z^2 is calicheamicin gamma dimethyl hydrazide or calicheamicin N-acetyl gamma dimethyl hydrazide.

12. A cytotoxic drug conjugate according to claim 9, wherein Z^3 is a monoclonal antibody which recognizes a glycoprotein antigen present on colon cancer cells and Z^2 is calicheamicin gamma dimethyl hydrazide or calicheamicin N-acetyl gamma dimethyl hydrazide.

13. A cytotoxic drug conjugate according to claim 9, wherein Z^3 is a monoclonal antibody which recognizes the IL2 receptor found on cells selected from the group consisting of activated and functionally mature T cells and abnormally activated leukemia cells and Z^2 is calicheamicin gamma dimethyl hydrazide or calicheamicin N-acetyl gamma dimethyl hydrazide.

14. A cytotoxic drug conjugate according to claim 9, wherein Z^3 is antibody h- or m-P67.6, h- or m-CT-M-01, h- or m-A33, or anti-Tac and Z^2 is calicheamicin gamma dimethyl hydrazide or calicheamicin N-acetyl gamma dimethyl hydrazide.

15. A cytotoxic drug conjugate according to claim 14 wherein Z^3 is antibody h-CT-M-01 and Z^2 is calicheamicin N-acetyl gamma dimethyl hydrazide.

16. A cytotoxic drug conjugate according to claim 14 wherein Z^3 is antibody h-P67.6 and Z^2 is calicheamicin N-acetyl gamma dimethyl hydrazide.

17. A cytotoxic drug conjugate according to claim 14, wherein Z^3 is antibody h-P67.6, Z^2 is calicheamicin N-acetyl gamma dimethyl hydrazide, Sp^1 is - O-, Alk^1 is C_3 alkylene, Ar is 1,4-phenylene, and Z^1 is C_1 alkyl.

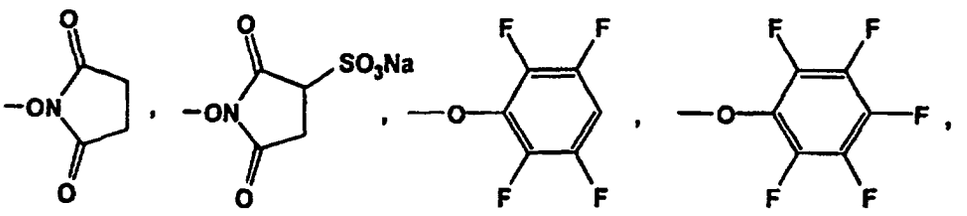
18. A cytotoxic drug conjugate according to claim 8 wherein Sp^1 is -O- or a bond, Alk^1 is a bond or branched or unbranched (C_1-C_{10}) alkylene chain, with the proviso that when Alk^1 is a bond, Sp^1 is a bond, Z^1 is (C_1-C_5) alkyl, and Z^2 is calicheamicin N-acetyl gamma dimethyl hydrazide.

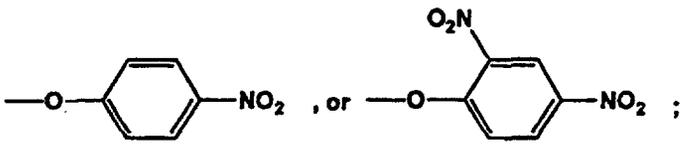
19. A compound of the formula

$$Z^3(CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=Z^2)$$

wherein

Z^3 is halogen, hydroxy, OM wherein M is a metal completing a salt, $-N_3$,





;

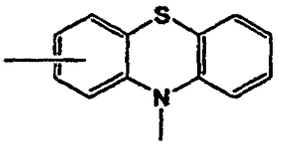
Alk^1 and Alk^2 are independently a bond or branched or unbranched (C_1-C_{10}) alkylene chain;

Sp^1 is a bond, -S-, -O-, -CONH-, -NHCO-, -NR'-, $-N(CH_2CH_2)_2N-$, or -X-Ar'-Y-$(CH_2)_n$-Z wherein X, Y, and Z are independently a bond, -NR'-, -S-. or -O-, with the proviso that when n = 0, then at least one of Y and Z must be a bond and Ar' is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, CO-OR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$, with the proviso that when Alk^1 is a bond, Sp^1 is a bond;

n is an integer from 0 to 5;

R' is a branched or unbranched (C_1-C_5) chain optionally substituted by one or two groups of -OH, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, (C_1-C_3) dialkylamino, or (C_1-C_3) trialkylammonium-A^- where A^- is a pharmaceutically acceptable anion completing a salt;

Ar is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, or COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ wherein n and R' are as defined above or a 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6-, or 2,7-naphthylidene or



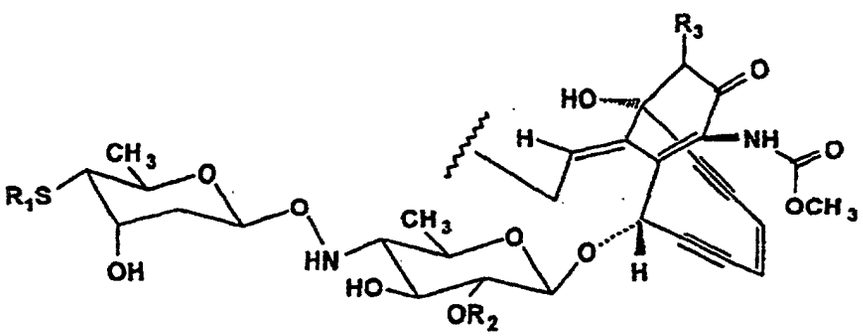
,

each naphthylidene or phenothiazine optionally substituted with one, two, three, or four groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ wherein n and R' are as defined above, with the proviso that when Ar is naphthylidene, Z^1 is not hydrogen and with the proviso that when Ar is phenothiazine, Sp^1 is a bond only connected to nitrogen;

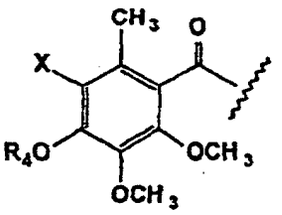
Sp^2 is a bond, -S-, or -O-, with the proviso that when Alk^2 is a bond, Sp^2 is a bond;

Z' is H, (C_1-C_5) alkyl, or phenyl optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ wherein n and R' are as defined above;

Z^2 is Q-Sp-S-S-W, wherein W is

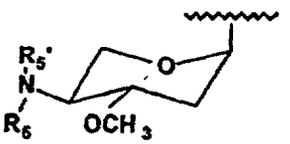


R_1 is



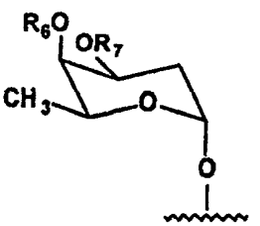
or CH_3;

R_2 is



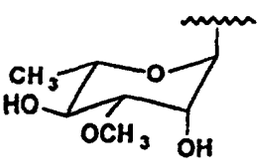
or H;

R_3 is



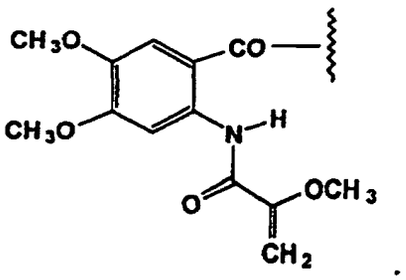
or H;

R_4 is



or H;

R_6 or R_7 is H or



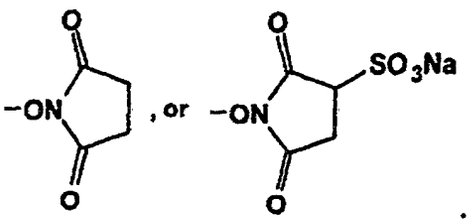
;

R_5 is $-CH_3$, $-C_2H_5$, or $-CH(CH_3)_2$; X is an iodine or bromine atom; R_5' is a hydrogen or the group RCO, wherein R is hydrogen, branched or unbranched (C_1 - C_{10}) alkyl or (C_1 - C_{10}) alkylene group, a (C_6-C_{11}) aryl group, a (C_6-C_{11}) aryl-alkyl (C_1 - C_5) group, or a heteroaryl or heteroaryl-alkyl (C_1 - C_5) group wherein heteroaryl is 2- or 3-furyl, 2- or 3-thienyl, 2-or 3-(N-methylpyrrolyl), 2-, 3-, or 4-pyridyl, 2-, 4-, or 5-(N-methylimidazolyl), 2-, 4-, or 5-oxazolyl, 2-, 3-, 5-, or 6-pyrimidinyl, 2-, 3-, 4-, 5-, 6-, 7-, or 8-quinolyl, or 1-, 3-, 4-, 5-, 6-, 7-, or 8-isoquinolyl, all aryl and heteroaryl optionally substituted by one or more hydroxy, amino, carboxy, halo, nitro, lower (C_1 - C_3) alkoxy, or lower (C_1 - C_5) thioalkoxy groups;

Sp is a straight or branched-chain divalent or trivalent (C_1 - C_{18}) radical, divalent or trivalent aryl or heteroaryl radical, divalent or trivalent (C_3 - C_{18}) cycloalkyl or heterocycloalkyl radical, divalent or trivalent aryl- or heteroaryl-alkyl (C_1 - C_{18}) radical, divalent or trivalent cycloalkyl- or heterocycloalkyl-alkyl (C_1 - C_{18}) radical or divalent or trivalent (C_2 - C_{18}) unsaturated alkyl radical, wherein heteroaryl is furyl, thienyl, N-methylpyrrolyl, pyridinyl, N-methylimidazolyl, oxazolyl, pyrimidinyl, quinolyl, isoquinolyl, N-methylcarbazoyl, aminocoumarinyl, or phenazinyl and wherein when Sp is a trivalent radical, Sp may be additionally substituted by lower (C_1 - C_5) dialkylamino, lower (C_1 - C_5) alkoxy, hydroxy, or lower (C_1 - C_5) alkylthio groups;

Q is =NHNCO-, =NHNCS-, =NHNCONH-, =NHNCSNH-, or =NO-.

20. A compound according to claim 19 wherein Z^3 is hydroxy,



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21. A compound according to claim 20 wherein Alk^2 is a branched or unbranched (C_1-C_{10}) alkylene chain and Z^1 is phenyl optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$.

22. A compound according to claim 20 wherein Alk^2 is a branched or unbranched (C_1-C_{10}) alkylene chain and Z^1 is H or (C_1-C_5) alkyl.

23. A compound according to claim 20 wherein Alk^2 and Sp^2 are together a bond and Z^1 is H or (C_1-C_5) alkyl.

24. A compound according to claim 20 wherein Alk^2 and Sp^2 are together a'bond and Z^1 is phenyl optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$.

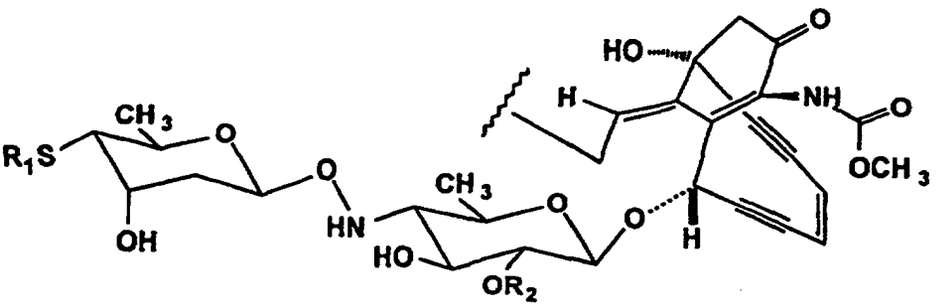
25. A compound according to claim 20, wherein Sp^1 is a bond, -S-, -O-, -CONH-, - NHCO-, or -NR', with the proviso that when Alk^1 is a bond, Sp^1 is a bond.

26. A compound according to claim 25 wherein Ar is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two,

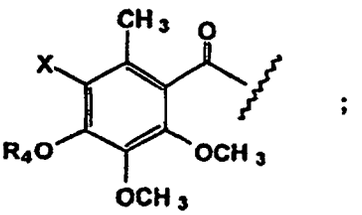
or three groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, or COOR', CONHR', O$(CH_2)_n$COOR', S$(CH_2)_n$COOR', O$(CH_2)_n$CONHR', or S$(CH_2)_n$CONHR' or a 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6-, or 2,7-naphthylidene each optionally substituted with one, two, three, or four groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, or COOR', CONHR', O$(CH_2)_n$COOR', S$(CH_2)_n$COOR', O$(CH_2)_n$CONHR', or S$(CH_2)_n$CONHR'.

27. A compound according to claim 26 wherein

Z^2 is Q-Sp-SS-W, wherein W is

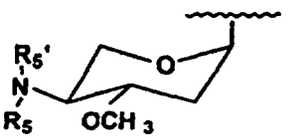


R_1 is



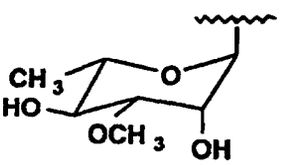
;

R_2 is



or H;

R_4 is



or H;

R_5, X, R_5', R, and Sp are as defined in claim 19; and

Q is =NHNCO-.

28. A compound according to claim 27 wherein Alk^2 and Sp^2 are together a bond, Ar is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, or COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$, and Z^1 is H or (C_1-C_5) alkyl.

29. A compound according to claim 28 wherein Sp^1 is -O-, Alk^1 is C_4 alkyl, Ar is 1,4-phenylene, and Z^1 is C_1 alkyl.

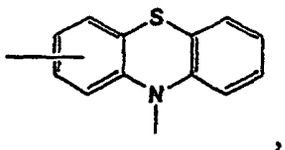
30. A compound according to claim 29 wherein Z^2 is calicheamicin gamma dimethyl hydrazide or calicheamicin N-acetyl gamma dimethyl hydrazide.

31. A compound of the formula:

$$Z^3CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=O$$

Wherein

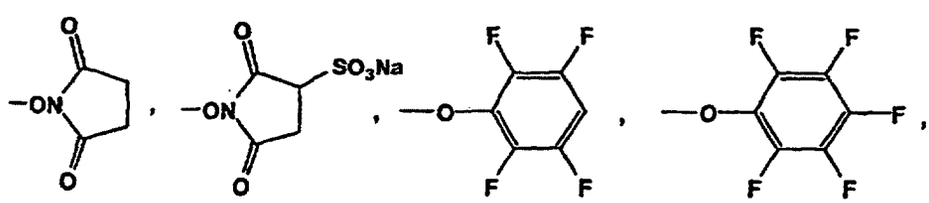
Ar is a 1,2-, 1,8-, or 2,7-naphthylidene or

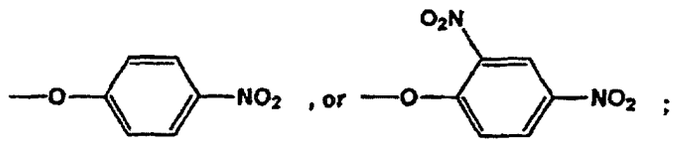


,

each naphthylidene or phenothiazine optionally substituted with one, two, three, or four groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, or COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ wherein n is an integer from 0 to 5 and R' is a branched or unbranched (C_1-C_5) chain optionally substituted by one or two groups of -OH, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, (C_1-C_3) dialkylamino, or (C_1-C_3) trialkylammonium - A^- where A^- is a pharmaceutically acceptable anion completing a salt, with the proviso that when Ar is phenothiazine, Sp^1 is a bond only connected to nitrogen;

Z^3 is halogen, hydroxy, OM wherein M is a metal completing a salt, $-N_3$,





; or

Alk^1 and Alk^2 are independently a bond or branched or unbranched (C_1-C_{10}) alkylene chain;

Sp^1 is a bond, -S-, -O-, -CONH-, -NHCO-, -NR'-, $-N(CH_2CH_2)_2N-$, or -X-Ar'-Y-$(CH_2)_n$-Z wherein X, Y, and Z are independently a bond, -NR'-, -S-, or -O-, with the proviso that when n = 0, then at least one of Y and Z must be a bond, and Ar' is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, CO-

OR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ wherein n and R' are as hereinbefore defined, with the proviso that when Alk^1 is a bond, then Sp^1 is a bond;

Sp^2 is a bond, -S-, or -O-, with the proviso that when Alk^2 is a bond, Sp^2 is a bond; and

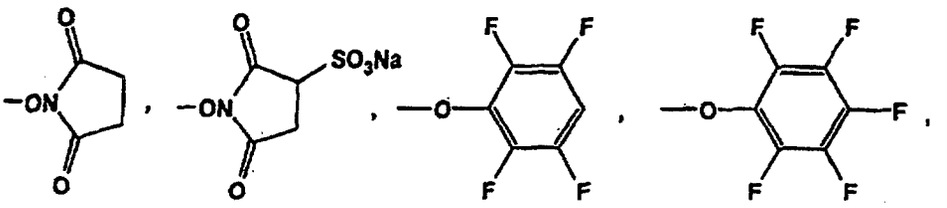
Z^1 is H, (C_1-C_5) alkyl, or phenyl optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ wherein n and R' are as hereinbefore defined, with the proviso that when Ar is naphthylidene, Z^1 is not hydrogen and with the proviso that when Alk^1 is a bond, Sp^2 is a bond, and Alk^2 is not a bond, then Z^1 is not C_1 alkyl.

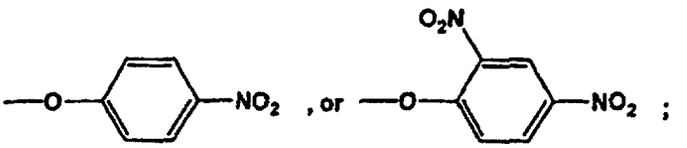
32. A compound of the formula:

$$Z^3CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=O$$

Wherein Ar is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, or COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ wherein n is an integer from 0 to 5 and R' is a branched or unbranched (C_1-C_5) chain optionally substituted by one or two groups of -OH, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, (C_1-C_3) dialkylamino, or (C_1-C_3) trialkylammonium - A- where A- is a pharmaceutically acceptable anion completing a salt;

Z^3 is halogen, hydroxy, OM wherein M is a metal completing a salt, $-N_3$,





Alk^1 and Alk^2 are, independently, a branched or unbranched (C_1-C_{10}) alkylene chain;

Sp^1 is a bond, -S-, -O-, -CONH-, -NHCO-, -NR'-, $-N(CH_2CH_2)_2N-$, or -X-Ar'-Y-$(CH_2)_n$-Z wherein X, Y, and Z are independently a bond, -NR'-, -S-, or -O-, with the proviso that when n = 0, then at least one of.Y and Z must be a bond, and Ar' is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$, wherein n and R' are as hereinbefore defined;

Sp^2 is a bond, -S-, or -O-, with the proviso that Sp^1 and Sp^2 are not both simultaneously a bond; and

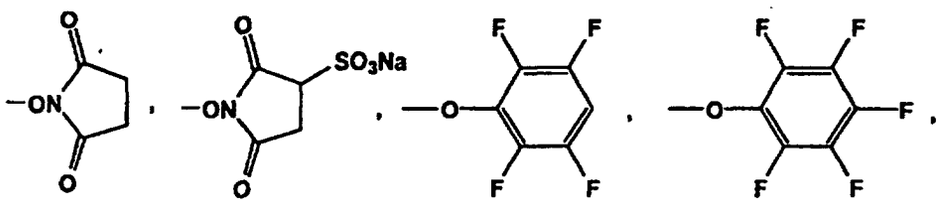
Z^1 is phenyl optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ wherein n and R' are as hereinbefore defined.

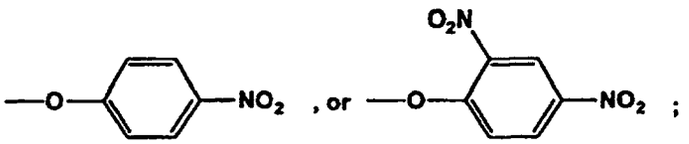
33. A compound of the formula:

$$Z^3CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=O$$

Wherein Ar is 1,2-, 1,3-, or 1,4-phenylene substituted with COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$ and optionally substituted with one or two groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, or nitro, wherein n is an integer from 0 to 5 and R' is a branched or unbranched (C_1-C_5) chain optionally substituted by one or two groups of -OH, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, (C_1-C_3) dialkylamino, or (C_1-C_3) trialkylammonium - A^- where A^- is a pharmaceutically acceptable anion completing a salt;

Z^3 is halogen, hydroxy, OM wherein M is a metal completing a salt, $-N_3$,





Alk^1 and Alk^2 are independently a bond or branched or unbranched (C_1-C_{10}) alkylene chain;

Sp^1 is a bond, -S-, -O-, -CONH-, -NHCO-, -NR'-, $-N(CH_2CH_2)_2N-$, or -X-Ar'-Y-$(CH_2)_n$-Z wherein X, Y, and Z are independently a bond, -NR'-, -S-, or -O-, with the proviso that when n = 0, then at least one of Y and Z must be a bond, and Ar' is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, CO-OR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$, with the proviso that when Alk^1 is a bond, Sp^1 is a bond wherein n and R' are as hereinbefore defined in (C), with the proviso that when Ar is 1,3- or 1,4- phenylene optionally substituted with one group of (C_1-C_6) alkyl or (C_1-C_5) alkoxy and Alk^2 is a bond, then Sp^1 is not a bond, -O- or -NHCO-;

Sp^2 is a bond, -S-, or -O-, with the proviso that when Alk^2 is a bond, Sp^2 is a bond; and

Z^1 is H or (C_1-C_5) alkyl.

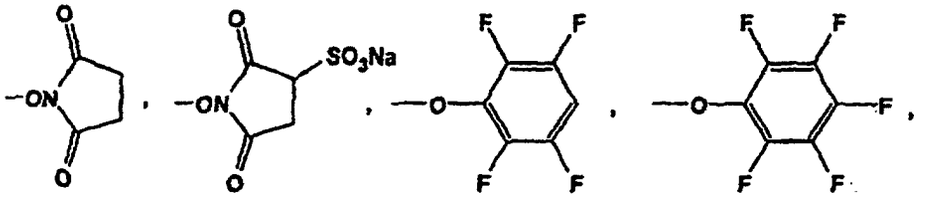
34. A compound of the formula:

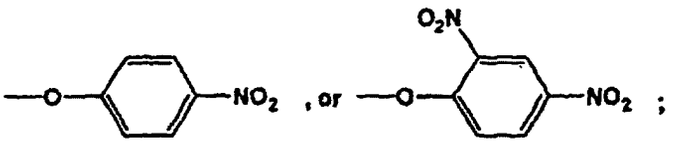
$$Z^3CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=O$$

Wherein

Ar is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, or nitro;

Z^3 is halogen, hydroxy, OM wherein M is a metal completing a salt, $-N_3$,





Alk^1 is a branched or unbranched (C_1-C_{10}) alkylene chain;
Alk^2 is a bond or branched or unbranched (C_1-C_{10}) alkylene chain;
Sp^1 is -CONH-;
Sp^2 is a bond, -S, or -O-, with the proviso that when Alk^2 is a bond, Sp^2 is a bond; and
Z^1 is H or (C_1-C_5) alkyl;

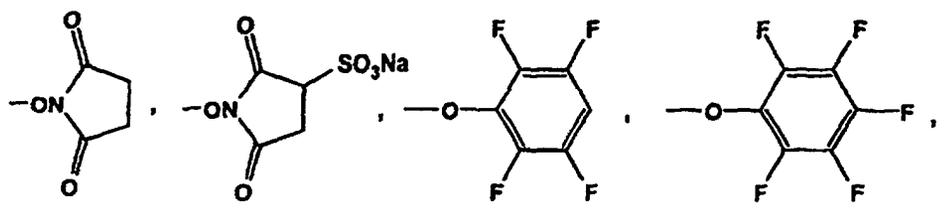
with the proviso that when Alk^1 is C_2, Alk^2 is a bond, and Z^1 is C_1, then Ar has at least one substituent, with the proviso that when Alk^1 is C_2, Z^1 is H, Alk^2 is a bond, and Ar is 1,4-phenylene then Ar has at least one substituent, and with the proviso that when Alk^1 is C_3, Z^1 is C_1, Alk^2 is a bond, and Ar is 1,4-phenylene, then Ar has at least one substituent.

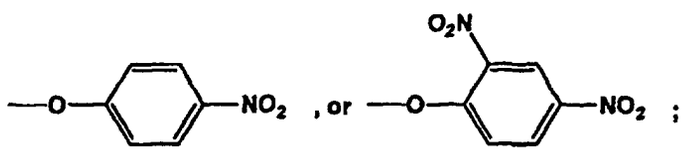
35. A compound of the formula:

$$Z^3CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=O$$

Wherein

Ar is 1,2-, 1,3- or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, or nitro;
Z^3 is halogen, hydroxy, OM wherein M is a metal completing a salt, $-N_3$,





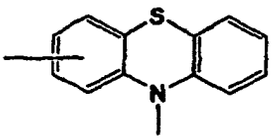
Alk^1 is a branched or unbranched (C_1-C_{10}) alkylene chain;
Alk^2 is a bond or a branched or unbranched (C_1-C_{10}) alkylene chain;
Sp^1 is a -S-, $-N(CH_2CH_2)_2N-$, or -X-Ar'-Y-$(CH_2)_n$-Z wherein X, Y, and Z are independently a bond, -NR'-, -S-, or -O-, with the proviso that when n = 0, then at least one of Y and Z must be a bond, and Ar' is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$, wherein n and R' are as hereinbefore defined;
Sp^2 is a bond, -S-, or -O-, with the proviso that when Alk^2 is a bond, Sp^2 is a bond; and
Z^1 is H or (C_1-C_5) alkyl.

36. A compound according to claim 31, wherein Z^1 is H or (C_1-C_5) alkyl and Sp^2 and Alk^2 are each a bond.

37. A compound according to claim 36, wherein Sp^1 is a bond, -S-, -O-, -CONH-, -NHCO-, -NR'-, or -N(CH_2CH_2)$_2$N- and Alk^1 is a branched or unbranched (C_1-C_{10}) alkylene chain.

38. A compound according to claim 37, wherein

Ar is an unsubstituted 1,2-, 1,8-, or 2,7-naphthylidene or



39. A compound according to claim 32, wherein Sp^1 is a bond, -S-, -O-, -CONH-, -NHCO-, -NR'-, or -N(CH_2CH_2)$_2$N-.

40. A compound according to claims 33, 34 or 35 wherein Alk^2 and Sp^2 are each a bond.

41. A compound according to claim 33, wherein Alk^1 is a branched or unbranched (C_1-C_{10}) alkylene chain and Sp^1 is a bond, -S-, -O-, -CONH-, -NHCO-, -NR'-, -N(CH_2CH_2)$_2$N- and Alk^2 and Sp^2 are each a bond.

42. A compound according to claim 39, wherein Ar is unsubstituted 1,2-, 1,3-, or 1,4-phenylene and Z^1 is unsubstituted phenyl.

43. A compound according to claim 41, wherein Ar is unsubstituted 1,2-, 1,3-, or 1,4-phenylene and Z^1 is (C_1-C_5) alkyl.

44. A compound according to claim 34, wherein Alk^2 and Sp^2 are each a bond, Ar is unsubstituted 1,2-, 1,3-, or 1,4-phenylene, and Z^1 is (C_1-C_5) alkyl.

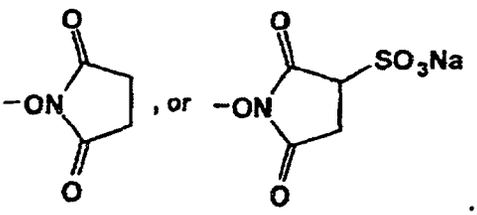
45. A compound according to claim 35, wherein Sp^1 is a -S- or -N(CH_2CH_2)$_2$N- and Alk^2 and Sp^2 are each a bond.

46. A compound according to claims 39, 41 or 45, wherein Ar is unsubstituted 1,2-, 1,3-, or 1,4-phenylene.

47. A compound according to claim 45, wherein Ar is unsubstituted 1,2-, 1,3-, or 1,4-phenylene and Z^1 is (C_1-C_5) alkyl.

48. A compound according to claim 34, wherein Ar is unsubstituted 1,2-, 1,3-, or 1,4-phenylene and Alk^2 and Sp^2 are each a bond.

49. A compound according to claims 38, 42, 43, 44, or 47, wherein Z^3 is hydroxy,



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50. A pharmaceutical composition for inhibiting the growth of cells, comprising an effective cell growth-inhibiting amount of the conjugate of claim 1 and a parenterally-administrable medium.

51. A pharmaceutical composition for inhibiting the growth of cells, comprising an effective cell growth-inhibiting amount of the conjugate of claim 15 or 16 and a parenterally-administrable medium.

52. A freeze-dried pharmaceutical composition for inhibiting the growth of cells, comprising a conjugate of claim 15 or

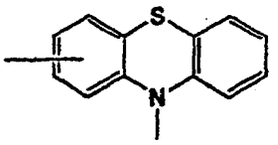
16 which is obtained by freeze-drying an approximately 1 mg/mL solution of the conjugate dissolved in about 5 mM sodium phosphate buffer at a pH of about 7.4 containing about 100 mM sodium chloride and about 100 mM sucrose.

53. A process for preparing the targeted derivatives of formula

$$Z^3(CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=Z^2)m$$

wherein

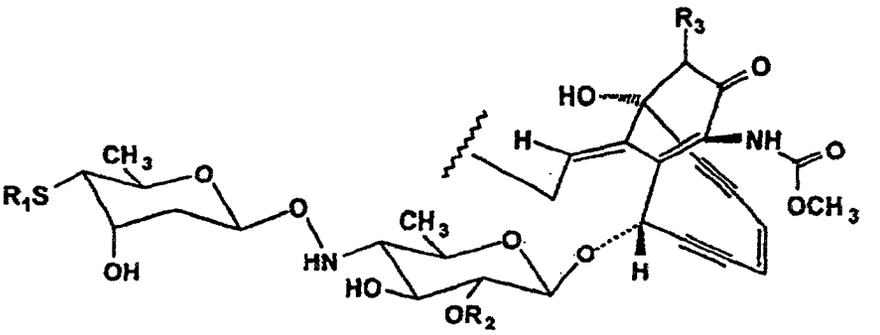
Z^3	is a protein selected from mono- and polyclonal antibodies, their antigen-recognizing fragments, and their chemically or genetically manipulated counterparts;
Alk^1 and Alk^2	are independently a bond or branched or unbranched (C_1-C_{10}) alkylene chain;
Sp^1	is a bond, -S-, -O-, -CONH-, -NHCO-, -NR'-, -N(CH_2CH_2)$_2$N-, or -X-Ar'-Y-(CH_2)$_n$-Z wherein X, Y, and Z are independently a bond, -NR'-, -S-, or -O-, with the proviso that when n = 0, then at least one of Y and Z must be a bond and Ar' is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, CO-OR', CONHR', O(CH_2)$_n$COOR', S(CH_2)$_n$COOR', O(CH_2)$_n$CONHR', or S(CH_2)$_n$CONHR', with the proviso that when Alk^1 is a bond, Sp^1 is a bond;
n	is an integer from 0 to 5;
R'	is a branched or unbranched (C_1-C_5) chain optionally substituted by one or two groups of -OH, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, (C_1-C_3) dialkylamino, or (C_1-C_3) trialkylammonium-A^- where A^- is a pharmaceutically acceptable anion completing a salt;
Ar	is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, or COOR', CONHR', O(CH_2)$_n$COOR', S(CH_2)$_n$COOR', O(CH_2)$_n$CONHR', or S(CH_2)$_n$CONHR' wherein n and R' are as defined above or a 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6-, or 2,7-naphthylidene or



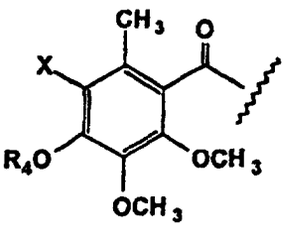
,

each naphthylidene or phenothiazine optionally substituted with one, two, three, or four groups of (C_1-C_6)alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, COOR', CONHR', O(CH_2)$_n$CO-OR', S(CH_2)$_n$COOR', O(CH_2)$_n$CONHR', or S(CH_2)$_n$CONHR' wherein n and R' are as defined above, with the proviso that when Ar is naphthylidene, Z^1 is not hydrogen and with the proviso that when Ar is phenothiazine, Sp^1 is a bond only connected to nitrogen;

Sp^2	is a bond, -S-, or -O-, with the proviso that when Alk^2 is a bond, Sp^2 is a bond;
Z^1	is H, (C_1-C_5) alkyl, or phenyl optionally substituted with one, two, or three groups of (C_1-C_5) alkyl, (C_1-C_4) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, COOR', CONHR', O(CH_2)$_n$COOR', S(CH_2)$_n$COOR', O(CH_2)$_n$CONHR', or S(CH_2)$_n$CONHR' wherein n and R' are as defined above;
Z^2	is Q-Sp-S-S-W, wherein W is

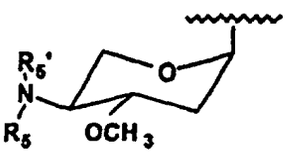


R_1 is



or CH_3;

R_2 is



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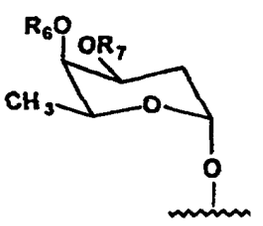
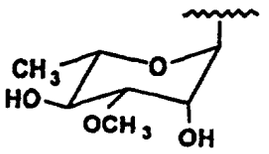
or H;

R_3 is



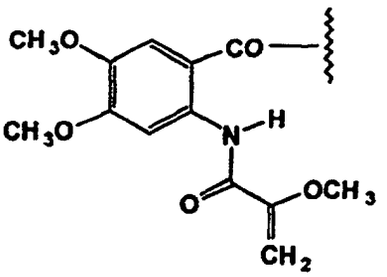
or H;

R_4 is



or H;

R_6 or R_7 is H or



;

R_5 is $-CH_3$, $-C_2H_5$, or $-CH(CH_3)_2$; X is an iodine or bromine atom; R_5' is a hydrogen or the group RCO, wherein R is hydrogen, branched or unbranched (C_1 - C_{10}) alkyl or (C_1 - C_{10}) alkylene group, a (C_6-C_{11}) aryl group, a (C_6-C_{11}) aryl-alkyl (C_1 - C_5) group, or a heteroaryl or heteroaryl-alkyl (C_1 - C_5) group wherein heteroaryl is 2- or 3-furyl, 2- or 3-thienyl, 2-or 3-(N-methylpyrrolyl), 2-, 3-, or 4-pyridyl, 2-, 4-, or 5-(N-methylimidazolyl), 2-, 4-, or 5-oxazolyl, 2-, 3-, 5-, or 6-pyrimidinyl, 2-, 3-, 4-, 5-, 6-, 7-, or 8-quinolyl, or 1-, 3-, 4-, 5-, 6-, 7-, or 8-isoquinolyl, all aryl and heteroaryl optionally substituted by one or more hydroxy, amino, carboxy, halo, nitro, lower (C_1 - C_3) alkoxy, or lower (C_1 - C_5) thioalkoxy groups;

Sp is a straight or branched-chain divalent or trivalent (C_1 - C_{18}) radical, divalent or trivalent aryl or heteroaryl radical, divalent or trivalent (C_3 - C_{18}) Cycloalkyl or heterocycloalkyl radical, divalent or trivalent aryl- or heteroaryl-alkyl (C_1 - C_{18}) radical, divalent or trivalent cycloalkyl- or heterocycloalkyl-alkyl (C_1 - C_{18}) radical or divalent or trivalent (C_2 - C_{18}) unsaturated alkyl radical, wherein heteroaryl is furyl, thienyl, N-methylpyrrolyl; pyridinyl, N-methylimidazolyl, oxazolyl, pyrimidinyl, quinolyl, isoquinolyl, N-methylcarbazoyl, amiocoumainyl, or phenazinyl and wherein when Sp is a trivalent radical, Sp may be additionally substituted by lower (C_1 - C_5) dialkylamino, lower (C_1 - C_5) alkoxy, hydroxy, or lower (C_1 - C_5) alkylthio groups;

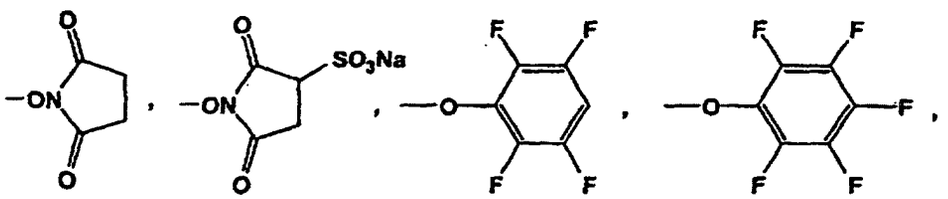
Q is =NHNCO-, =NHNCS-, =NHNCONH-, =NHNCSNH-, or =NO-; and

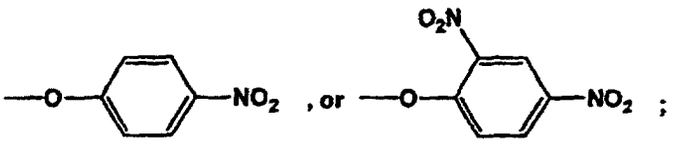
m is from about 0.1 to 15;

comprising reacting a compound of formula

$$Z^{3'}CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=Z^2$$

wherein Alk^1, Sp^1, Ar, Sp^2, Alk^2, Z^1, and $Z^2$are as defined above; and $Z^{3'}$ is





with a carrier Z^3, wherein Z^3 is a protein selected from mono- and polyclonal antibodies, their antigen-recognizing fragments, and their chemically or genetically manipulated counterparts, in an aqueous, buffered solution at a pH of between 6.5 and 9.0 and a temperature of 4° to 40°C for 1 to 48 hours to generate the targeted derivatives of formula

$$Z^3(CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=Z^2)m$$

defined above.

54. A process as claimed in claim 53 wherein the compound of formula

$$Z^{3'}CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=Z^2$$

is generated by:

(a) reacting H_2Z^2 with a compound of formula

$$HOCO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=O,$$

in an alcoholic solvent with a boiling point of less than about 100°C in the presence of about 5% acetic acid or a carboxylic acid catalyst at about 20° to 70°C for about 1 to 24 hours, wherein Alk^1 and Alk^2, Sp^1, n, R^1, Sp^2, Z^1, and Ar are as defined in claim 53, to produce an intermediate of formula

$$HOCO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=Z^2,$$

wherein Alk^1, Sp^1, Ar, Sp^2, Alk^2, Z^1, and Z^2 are as defined in claim 53;
(b) isolating the intermediate of step (a); and
(c) reacting the isolated intermediate of step (b) with N-hydroxysuccinimide, 2, 3, 5, 6-tetrafluorophenol, pentafluorophenol, 4-nitrophenol, 2,4-dinitrophenol, or N-hydroxysulfosuccinimide in the presence of DCC, EDCI, or other carbodiimide in an inert organic solvent such as acetonitrile or acetonitrile containing 5-50% DMF.

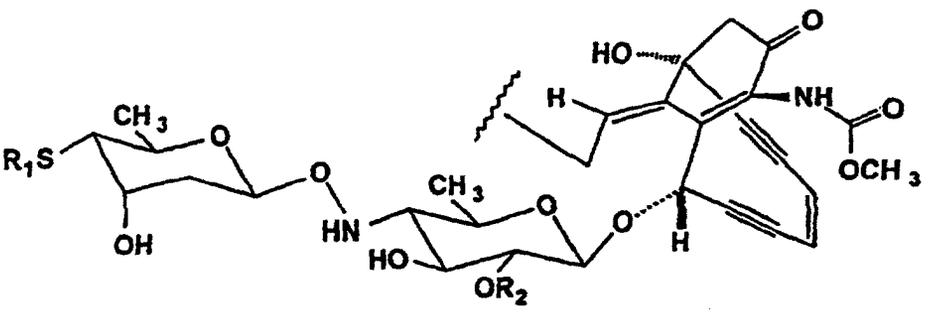
55. The process of claim 53 or claim 54 wherein Alk^2 and Sp^2 are together a bond and Z^1 is H or (C_1-C_5) alkyl.

56. The process of claim 54 or claim 55, wherein Sp^1 is a bond, -S-, -O-, -CONH-, -NHCO-, or -NR', with the proviso that when Sp^1 is a bond, Alk^1 is a bond.

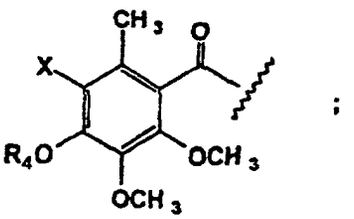
57. The process of claim 54 or claim 55, wherein Ar is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, or COOR', CONHR', O$(CH_2)_n$COOR', S$(CH_2)_n$COOR', O$(CH_2)_n$CONHR', or S$(CH_2)_n$CONHR' or a 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6-, or 2,7-naphthylidene, each optionally substituted with one, two, three, or four groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, COOR', CONHR', O$(CH_2)_n$COOR', S$(CH_2)_n$COOR', O$(CH_2)_n$CONHR', or S$(CH_2)_n$CONHR'.

58. The process of claim 54 or claim 55, wherein a covalent bond to the Z^3 protein is an amide formed from a reaction with the lysine side chains of the Z^3 protein.

59. The process of claim 54 or claim 55, wherein Z^2 is Q-Sp-S-S-W and W is

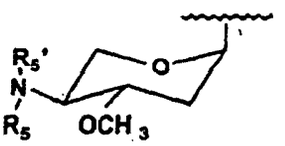


R_1 is



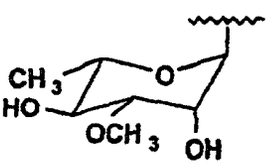
;

R_2 is



or H;

R_4 is



or H;

R_5, X, R_5', R, and Sp are as defined in claim 49, and Q is =NHNCO-.

60. The process of claim 54, wherein the alcoholic solvent of step (a) is methanol; the carboxylic acid catalyst of step (a) is 5% acetic acid; the isolated intermediate of step (b) is reacted in step (c) with N-hydroxysuccinimide in the presence of EDCI in acetonitrile; and the aqueous buffered solution of step (d) is phosphate buffer having a pH of 7.4 to 8.0.

61. The process of claim 60, wherein Z^1 is (C_1-C_5) alkyl.

62. The process of claim 61, wherein Ar is 1,2-, 1,3-, or 1,4-phenylene optionally substituted with one, two, or three

groups of (C_1-C_6) alkyl, (C_1-C_5) alkoxy, (C_1-C_4) thioalkoxy, halogen, nitro, or COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$, or $S(CH_2)_nCONHR'$.

63. The process of claim 62, wherein Sp^1 is -O-, Alk^1 is C_4 alkylene, Ar is 1,4-phenylene, and Z^1 is C_1 alkyl.

64. The process of claim 63, wherein Z^3 is antibody h-P67.6, h-CT-M-01, m-CT-M-01, h-A33, m-A33 or anti-Tac.

65. The process of claim 64, wherein Z^2 is calicheamicin gamma dimethyl hydrazide or calicheamicin N-acetyl gamma dimethyl hydrazide.

66. The process of claim 65, wherein Z^3 is antibody h-CT-M-01 and Z^2 is calicheamicin N-acetyl gamma dimethyl hydrazide.

67. The process of claim 65, wherein Z^3 is antibody h-P67.6 and Z^2 is calicheamicin N-acetyl gamma dimethyl hydrazide.

68. The process of claim 57, wherein Sp^1 is -O- or a bond; Alk^1 is a bond or branched or unbranched (C_1-C_{10}) alkylene chain, with the proviso that when Alk^1 is a bond, Sp^1 is a bond; Z^1 is (C_1-C_5) alkyl; and Z^2 is calicheamicin N-acetyl gamma dimethyl hydrazide.

69. A conjugate as claimed in any one of claims 1 to 18 for use to control the growth of an undesirable cell in a mammal.

70. A compound for use as claimed in claim 69 wherein the undesirable cell is a cancer cell.

71. A compound for use as claimed in claim 70 wherein the cancer is breast, lung or ovarian cancer or leukemia.

72. The use of a conjugate as claimed in any one of claims 1 to 18 in the manufacture of a medicament for controlling the growth of an undesirable cell in a mammal.

73. A use as claimed in claim 72 wherein the undesirable cell is a cancer cell.

74. A use as claimed in claim 73 wherein the cancer is breast, lung or ovarian cancer or leukemia.

Patentansprüche

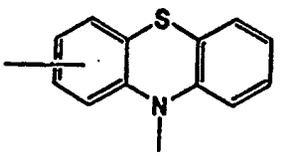
1. Cytotoxisches Arzneimittelkonjugat der Formel:

$$Z^3\ (CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=Z^2)_m,$$

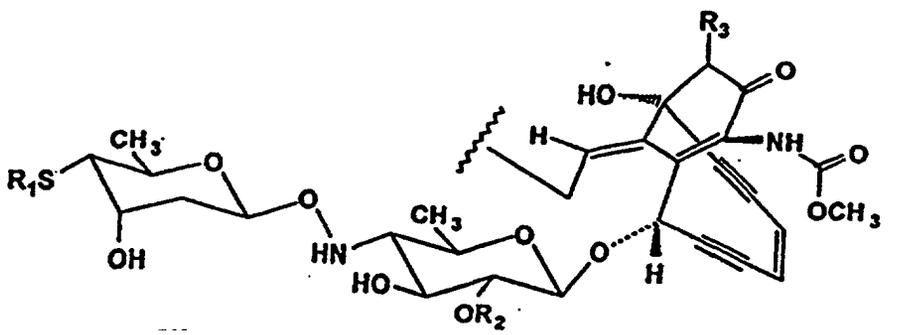
worin

Z^3 ein Protein darstellt, ausgewählt aus mono- und polyklonalen Antikörpern, ihren Antigen-erkennenden Fragmenten und ihren chemisch oder genetisch manipulierten Gegenstücken, und Wachstumsfaktoren und ihren chemisch oder genetisch manipulierten Gegenstücken, worin eine kovalente Bindung zu dem Protein ein Amid ist, gebildet aus Umsetzung mit "m"-Lysin-Seitenketten, oder ein Steroid, worin die kovalente Bindung zu dem Steroid ein Amid oder ein Ester ist;
Alk^1 und Alk^2 unabhängig eine Bindung oder verzweigte oder unverzweigte (C_1-C_{10})-Alkylenkette darstellen;
Sp^1 eine Bindung, -S-, -O-, -CONH-, -NHCO-, -NR'-, $-N(CH_2CH_2)_2N-$ oder -X-Ar'-Y-$(CH_2)_n$-Z darstellt, worin X, Y und Z unabhängig eine Bindung, -NR'-, -S- oder -O- darstellen, unter der Voraussetzung, dass wenn n = 0, dann muss mindestens eins von Y und Z eine Bindung darstellen und Ar' steht für 1,2-, 1,3-oder 1,4-Phenylen, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von (C_1-C_5)-Alkyl, (C_1-C_4)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$, unter der Voraussetzung, dass wenn Alk^1 eine Bindung darstellt, Sp^1 eine Bindung darstellt;
n eine ganze Zahl von 0 bis 5 darstellt;
R' eine verzweigte oder unverzweigte (C_1-C_5)-Kette darstellt, gegebenenfalls substituiert durch eine oder zwei Gruppen von -OH, (C_1-C_4)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro, (C_1-C_3)-Dialkylamino oder (C_1-C_3)-Tri-

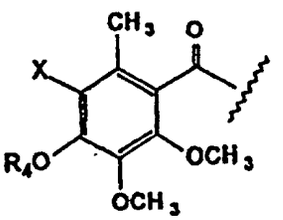
alkylammonium-A^-, worin A^- ein pharmazeutisch annehmbares Anion darstellt, welches ein Salz komplettiert;
Ar für 1,2-, 1,3- oder 1,4-Phenylen steht, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von $(C_1$-$C_6)$-Alkyl, $(C_1$-$C_5)$-Alkoxy, $(C_1$-$C_4)$-Thioalkoxy, Halogen, Nitro oder COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$, worin n und R' wie oben definiert sind, oder ein 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6- oder 2,7-Naphthyliden oder



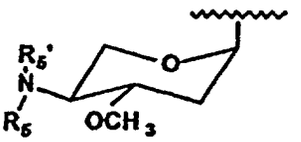
jedes Naphthyliden oder Phenothiazin gegebenenfalls substituiert mit einer, zwei, drei oder vier Gruppen von $(C_1$-$C_6)$-Alkyl, $(C_1$-$C_5)$-Alkoxy, $(C_1$-$C_4)$-Thioalkoxy, Halogen, Nitro oder COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$, worin n und R' wie oben definiert sind, unter der Voraussetzung, dass wenn Ar für Naphthyliden steht, Z^1 nicht für Wasserstoff steht, und unter der Voraussetzung, dass wenn Ar für Phenothiazin steht, Sp^1 eine nur an Stickstoff gebundene Bindung darstellt;
Sp^2 eine Bindung, -S- oder -O- darstellt, unter der Voraussetzung, dass wenn Alk^2 eine Bindung darstellt, Sp^2 eine Bindung darstellt;
Z^1 für H, $(C_1$-$C_5)$-Alkyl oder Phenyl steht, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von $(C_1$-$C_5)$-Alkyl, $(C_1$-$C_4)$-Alkoxy, $(C_1$-$C_4)$-Thioalkoxy, Halogen, Nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$, worin n und R' wie oben definiert sind;
Z^2 für O-Sp-S-S-W steht worin W für



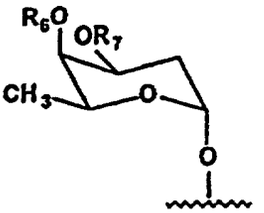
steht,
R_1



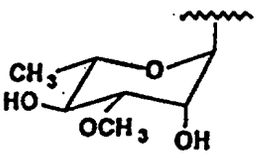
oder CH_3 darstellt;
R_2



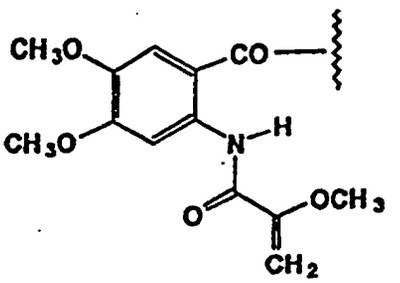
oder H darstellt;
R_3



oder H darstellt;
R_4



oder H darstellt,
R_6 oder R_7 für H oder



stehen;
R_5 für $-CH_3$, $-C_2H_5$ oder $-CH(CH_3)_2$ steht; X ein Iod- oder Bromatom darstellt; R_5' ein Wasserstoff oder die Gruppe RCO darstellt, worin R für Wasserstoff, verzweigtes oder unverzweigtes (C_1-C_{10})-Alkyl oder (C_1-C_{10})-Alkylengruppe, eine (C_6-C_{11})-Arylgruppe, eine (C_6-C_{11})-Arylalkyl(C_1-C_5)-gruppe oder eine Heteroaryl- oder Heteroaryl-alkyl-(C_1-C_5)-gruppe steht, worin Heteroaryl für 2-oder 3-Furyl, 2- oder 3-Thienyl, 2- oder 3-(N-Methylpyrrolyl), 2-, 3- oder 4-Pyridyl, 2-, 4- oder 5-(N-Methylimidazolyl), 2-, 4-oder 5-Oxazolyl, 2-, 3-, 5- oder 6-Pyrimidinyl, 2-, 3-, 4-, 5-, 6-, 7- oder 8-Chinolyl oder 1-, 3-, 4-, 5-, 6-, 7- oder 8-Isochinolyl steht, alles Aryl

und Heteroaryl gegebenenfalls substituiert durch eine oder mehrere Hydroxy-, Amirio-, Carboxy-, Halogen-, Nitro-, nied. -(C_1-C_3)-Alkoxy oder nied. -(C_1-C_5)-Thioalkoxygruppen;
Sp einen grad- oder verzweigtkettigen bivalenten oder trivalenten (C_1-C_{18})-Rest, bivalenten oder trivalenten Aryl- oder Heteroarylrest, bivalenten oder trivalenten (C_3-C_{18})-Cycloalkyl- oder Heterocycloalkylrest, bivalenten oder trivalenten Aryl- oder Heteroaryl-alkyl-(C_1-C_{18})-rest, bivalenten oder trivalenten Cycloalkyl- oder Heterocycloalkyl-alkyl-(C_1-C_{18})-rest oder bivalenten oder trivalenten (C_2-C_{18}) ungesättigten Alkylrest darstellt, worin Heteroaryl für Furyl, Thienyl, N-Methylpyrrolyl, Pyridinyl, N-Methylimidazolyl, Oxazolyl, Pyrimidinyl, Chinolyl, Isochinolyl, N-Methylcarbazoyl, Aminocumarinyl oder Phenazinyl steht und worin, wenn Sp einen trivalenten Rest darstellt, Sp zusätzlich substituiert sein kann durch nied.-(C_1-C_5)-Dialkylamino-, nied.-(C_1-C_5)-Alkoxy-, Hydroxy- oder nied.-(C_1-C_5)-Alkylthiogruppen; und
Q für =NHNCO-, =NHNCS-, =NHNCONH-, =NHNCSNH- oder =NHOsteht,
m von etwa 0,1 bis 15 ist.

2. Cytotoxisches Arzneimittelkonjugat gemäß Anspruch 1, worin Alk^2 eine verzweigte oder unverzweigte (C_1-C_{10})-Alkylenkette darstellt und Z^1 für Phenyl steht, gegebenenfalls substituiert durch eine, zwei oder drei Gruppen von (C_1-C_5)-Alkyl, (C_1-C_4)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$, worin n und R' wie in Anspruch 1 definiert sind.

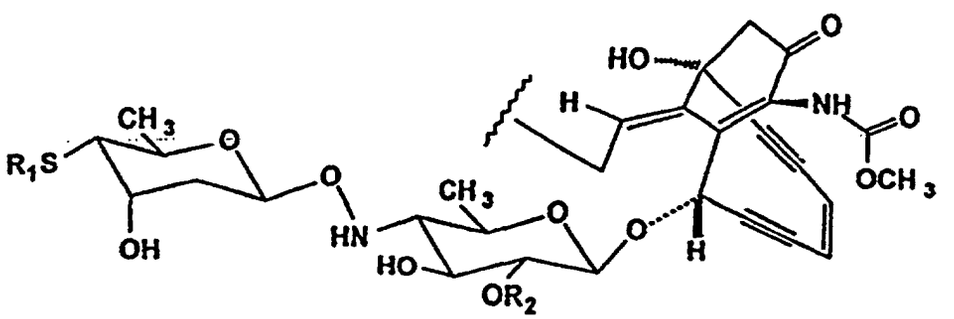
3. Cytotoxisches Arzneimittelkonjugat gemäß Anspruch 1, worin Alk^2 und Sp^2 zusammen eine Bindung darstellen und Z^1 für H oder (C_1-C_5)-Alkyl steht.

4. Cytotoxisches Arzneimittelkonjugat gemäß Anspruch 1, worin Sp^1 für ein Bindung, -S-, -O-, -CONH-, -NHCO- oder -NR', steht, worin R' wie in Anspruch 1 definiert ist, unter der Voraussetzung, dass wenn Alk^1 eine Bindung darstellt, Sp^1 eine Bindung darstellt.

5. Cytotoxisches Arzneimittelkonjugat gemäß Anspruch 4, worin Ar für 1,2-, 1,3- oder 1,4-Phenylen steht, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von (C_1-C_6)-Alkyl, (C_1-C_5)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro oder COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$, worin n und R' wie in Anspruch 1 definiert sind, oder Ar für ein 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6- oder 2,7-Naphthyliden steht, jedes gegebenenfalls substituiert mit einer, zwei, drei oder vier Gruppen von (C_1-C_6)-Alkyl, (C_1-C_5)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro oder COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$, worin n und R' wie in Anspruch 1 definiert sind.

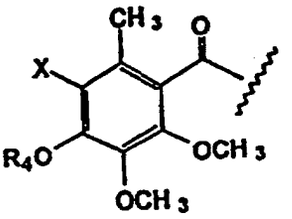
6. Cytotoxisches Arzneimittelkonjugat gemäß Anspruch 5, worin Z^3 ein Protein darstellt, ausgewählt aus mono- und polyklonalen Antikörpern, ihren Antigen-erkennenden Fragmenten und ihren chemisch oder genetisch manipulierten Gegenstücken, und Wachstumsfaktoren und ihren chemisch oder genetisch manipulierten Gegenstücken, worin eine kovalente Bindung zu dem Protein ein Amid ist, gebildet aus Umsetzung mit "m"-Lysin-Seitenketten.

7. Cytotoxisches Arzneimittelkonjugat gemäß Anspruch 6,worin Z^2 für Q-Sp-S-S-W steht, worin W für

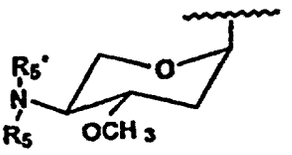


steht,

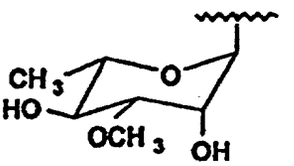
R_1



darstellt;
R_2



oder H darstellt;
R_4



oder H darstellt,
R_5, X, R_5', R und Sp wie in Anspruch 1 definiert sind; und Q für =NHNCO- steht.

8. Cytotoxisches Arzneimittelkonjugat gemäß Anspruch 7, worin Ar für 1,2-, 1,3- oder 1,4-Phenylen steht, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von (C_1-C_6)-Alkyl, (C_1-C_5)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro oder COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$, worin n und R' wie in Anspruch 1 definiert sind, Alk^2 und Sp^2 zusammen eine Bindung darstellen und Z^1 für H oder (C_1-C_5)-Alkyl steht.

9. Cytotoxisches Arzneimittelkonjugat gemäß Anspruch 8, worin Sp^1 für -O- oder eine Bindung steht, Alk^1 für C_1 bis C_6 Alkylen steht, Ar für 1,3- oder 1,4-Phenylen steht, gegebenenfalls substituiert mit einer oder zwei Gruppen von (C_1-C_3)-Alkyl, (C_1-C_3)-Alkoxy, Halogen, Nitro, COOR' oder CONHR', worin R' wie in Anspruch 1 definiert ist, und Z^1 für (C_1-C_3)-Alkyl steht.

10. Cytotoxisches Arzneimittelkonjugat gemäß Anspruch 9, worin Z^3 einen monoklonalen Antikörper darstellt, welcher das CD33-Antigen erkennt, und Z^2 für Calicheamicin-gamma-dimethylhydrazid oder Calicheamicin-N-acetyl-gamma-dimethylhydrazid steht.

11. Cytotoxisches Arzneimittelkonjugat gemäß Anspruch 9, worin Z^3 einen monoklonalen Antikörper darstellt, welcher das polyepitheliale Mucin-Antigen erkennt, und Z^2 für Calicheamicin-gamma-dimethylhydrazid oder Calicheamicin-N-acetyl-gamma-dimethylhydrazid steht.

12. Cytotoxisches Arzneimittelkonjugat gemäß Anspruch 9, worin Z^3 einen monoklonalen Antikörper darstellt, welcher ein Glycoprotein-Antigen erkennt, welches auf Dickdarmkrebszellen vorhan den ist, und Z^2 für Calicheamicin-gamma-dimethylhydrazid oder Calicheamicin-N-acetyl-gamma-dimethylhydrazid steht.

13. Cytotoxisches Arzneimittelkonjugat gemäß Anspruch 9, worin Z^3 einen monoklonalen Antikörper darstellt, welcher den IL2-Rezeptor erkennt, welcher auf Zellen gefunden wird, welche ausgewählt werden aus der Gruppe, bestehend aus aktivierten und funktionell reifen T-Zellen und abnormal aktivierten Leukämiezellen, und Z^2 für Calicheamicin-gamma-dimethylhydrazid oder Calicheamicin-N-acetyl-gamma-dimethylhydrazid steht.

14. Cytotoxisches Arzneimittelkonjugat gemäß Anspruch 9, worin Z^3 Antikörper h- oder m-P67.6, h- oder m-CT-M-01, h- oder m-A33 oder Anti-Tac darstellt, und Z^2 für Calicheamicin-gamma-dimethylhydrazid oder Calicheamicin-N-acetyl-gamma-dimethylhydrazid steht.

15. Cytotoxisches Arzneimittelkonjugat gemäß Anspruch 14, worin Z^3 Antikörper h-CT-M-01 darstellt und Z^2 für Calicheamicin-N-acetyl-gamma-dimethylhydrazid steht.

16. Cytotoxisches Arzneimittelkonjugat gemäß Anspruch 14, worin Z^3 Antikörper h-P67.6 darstellt und Z^2 für Calicheamicin-N-acetyl-gamma-dimethylhydrazid steht.

17. Cytotoxisches Arzneimittelkonjugat gemäß Anspruch 14, worin Z^3 Antikörper h-P67.6 darstellt, Z^2 für Calicheamicin-N-acetyl-gamma-dimethylhydrazid steht, Sp^1 für -O- steht, Alk^1 für C_3-Alkylen steht, Ar für 1,4-Phenylen steht und Z^1 für C_1-Alkyl steht.

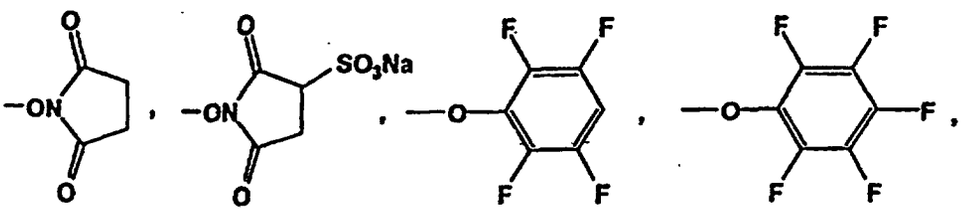
18. Cytotoxisches Arzneimittelkonjugat gemäß Anspruch 8, worin Sp^1 für -O- oder eine Bindung steht, Alk^1 für eine Bindung oder verzweigte oder unverzweigte (C_1-C_{10})-Alkylenkette steht, unter der Voraussetzung, dass wenn Alk^1 eine Bindung darstellt, Sp^1 eine Bindung darstellt, Z^1 für (C_1-C_5)-Alkyl steht und Z^2 für Calicheamicin-N-acetyl-gamma-dimethylhydrazid steht.

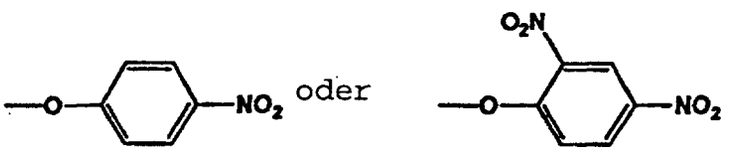
19. Verbindung der Formel

$$Z^3\ (CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=Z^2),$$

worin

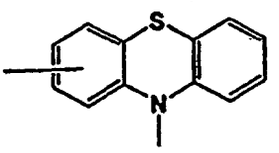
Z^3 Halogen, Hydroxy, OM worin M für ein Metall steht, welches ein Salz komplettiert, $-N_3$,



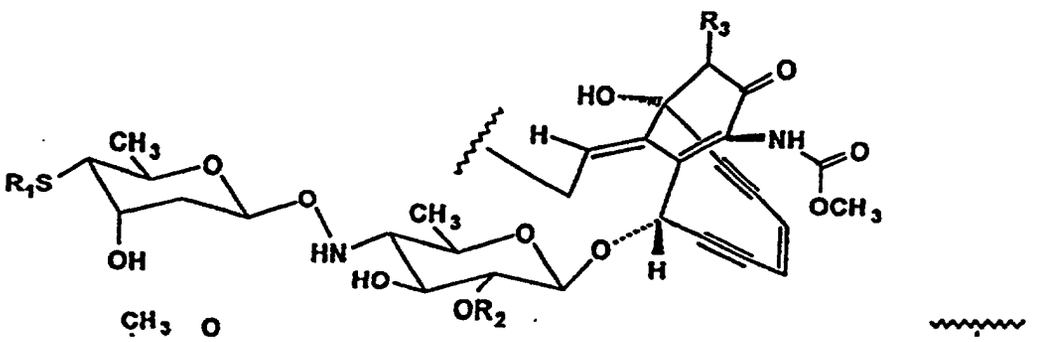


darstellt;
Alk^1 und Alk^2 unabhängig eine Bindung oder verzweigte oder unverzweigte (C_1-C_{10})-Alkylenkette sind;
Sp^1 eine Bindung, -S-, -O-, -CONH-, -NHCO-, -NR'-, $-N(CH_2CH_2)_2N-$ oder -X-Ar'-Y-$(CH_2)_n$-Z darstellt, worin X, Y und Z unabhängig eine Bindung, -NR'-, -S- oder -O- darstellen, unter der Voraussetzung, dass wenn n = 0, dann muss mindestens eins von Y und Z eine Bindung darstellen und AR' steht für 1,2-, 1,3-oder 1,4-Phenylen, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von (C_1-C_5)-Alkyl, (C_1-C_4)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$, unter der Voraussetzung, dass wenn Alk^1 eine Bindung darstellt, Sp^1 eine Bindung darstellt;

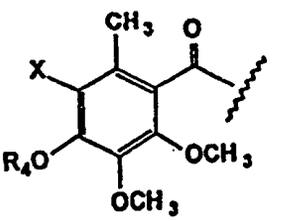
n eine ganze Zahl von 0 bis 5 darstellt;
R' eine verzweigte oder unverzweigte (C_1-C_5)-Kette darstellt, gegebenenfalls substituiert durch eine oder zwei Gruppen von -OH, (C_1-C_4)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro, (C_1-C_3)-Dialkylamino oder (C_1-C_3)-Trialkylammonium -A^-, worin A^- ein pharmazeutisch annehmbares Anion darstellt, welches ein Salz komplettiert;
Ar für 1,2-, 1,3- oder 1,4-Phenylen steht, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von (C_1-C_6)-Alkyl, (C_1-C_5) -Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro oder COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$, worin n und R' wie oben definiert sind, oder ein 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6- oder 2,7-Naphthyliden oder



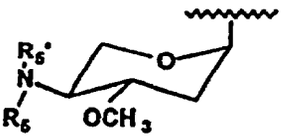
jedes Naphthyliden oder Phenothiazin gegebenenfalls substituiert mit einer, zwei, drei oder vier Gruppen von (C_1-C_6)-Alkyl, (C_1-C_5)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$, worin n und R' wie oben definiert sind, unter der Voraussetzung, dass wenn Ar für Naphthyliden steht, Z^1 nicht für Wasserstoff steht, und unter der Voraussetzung, dass wenn Ar für Phenothiazin steht, Sp^1 eine nur mit Stickstoff verbundene Bindung darstellt;
Sp^2 eine Bindung, -S- oder -O- darstellt, unter der Voraussetzung, dass wenn Alk^2 eine Bindung darstellt, Sp^2 eine Bindung darstellt;
Z^1 für H, (C_1-C_5)-Alkyl steht oder Phenyl, gegebenenfalls substituiert mit ein, zwei oder drei Gruppen von (C_1-C_5)-Alkyl, (C_1-C_4)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$ steht, worin n und R' wie oben definiert sind;
Z^2 für Q-Sp-S-S-W steht, worin W für steht,



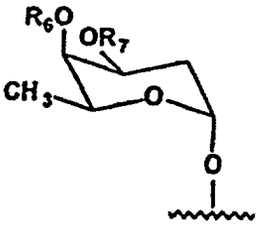
steht,
R_1



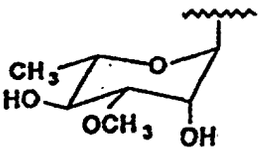
oder CH_3 darstellt;
R_2



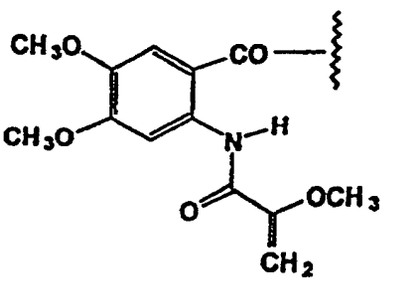
oder H darstellt;
R_3



oder H darstellt;
R_4



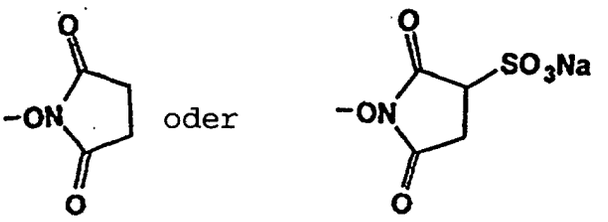
oder H darstellt,
R_6 oder R_7 für H oder



stehen;
R^5 für $-CH_3$, $-C_2H_5$ oder $-CH(CH_3)_2$ steht; X ein Iod- oder Bromatom darstellt; R_5' ein Wasserstoff oder die Gruppe RCO darstellt, worin R für Wasserstoff, verzweigtes oder unverzweigtes. (C_1-C_{10})-Alkyl oder (C_1-C_{10}) -Alkylengruppe, eine (C_6-C_{11})-Arylgruppe, eine (C_6-C_{11})-Arylalkyl(C_1-C_5)-gruppe oder eine Heteroaryl- oder Heteroaryl-alkyl-(C_1-C_5)-gruppe steht, worin Heteroaryl für 2-oder 3-Furyl, 2- oder 3-Thienyl, 2- oder 3-(N-Methylpyrrolyl), 2-, 3- oder 4-Pyridyl, 2-, 4- oder 5-(N-Methylimidazolyl), 2-, 4-oder 5-Oxazolyl, 2-, 3-, 5- oder 6-Pyrimidinyl, 2-, 3-, 4-, 5-, 6-, 7- oder 8-Chinolyl oder 1-, 3-, 4-, 5-, 6-, 7- oder 8-Isochinolyl steht, alles Aryl und Heteroaryl gegebenenfalls substituiert durch eine oder mehrere Hydroxy-, Amino-, Carboxy-, Halogen-, Nitro-, nied. -(C_1-C_3)-Alkoxy oder nied.-(C_1-C_5) -Thioalkoxygruppen;
Sp einen grad- oder verzweigtkettigen bivalenten oder trivalenten (C_1-C_{18})-Rest, bivalenten oder trivalenten

Aryl- oder Heteroarylrest, bivalenten oder trivalenten (C_3-C_{18})-Cycloalkyl oder Heterocycloalkylrest, bivalenten oder trivalenten Aryl- oder Heteroaryl-alkyl-(C_1-C_{18})-rest, bivalenten oder trivalenten Cycloalkyl- oder Heterocycloalkyl-alkyl-(C_1-C_{18})-rest oder bivalenten oder trivalenten (C_2-C_{18}) ungesättigten Alkylrest darstellt, worin Heteroaryl für Furyl, Thienyl, N-Methylpyrrolyl, Pyridinyl, N-Methylimidazolyl, Oxazolyl, Pyrimidinyl, Chinolyl, Isochinolyl, N-Methylcarbazoyl, Aminocumarinyl oder Phenazinyl steht und worin, wenn Sp einen trivalenten Rest darstellt, Sp zusätzlich substituiert sein kann durch nied.-(C_1-C_5)-Dialkylamino-, nied.-(C_1-C_5)-Alkoxy-, Hydroxy- oder nied-(C_1-C_5)-Alkylthiogruppen;
Q für =NHNCO-, =NHNCS-, =NHNCONH-, =NHNCSNH- oder =NOsteht.

20. Verbindung gemäß Anspruch 19, worin Z^3 für Hydroxy,



steht.

21. Verbindung gemäß Anspruch 20, worin Alk^2 eine verzweigte oder unverzweigte (C_1-C_{10})-Alkylenkette darstellt und Z^1 für Phenyl steht, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von (C_1-C_5)-Alkyl, (C_1-C_4)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$.

22. Verbindung gemäß Anspruch 20, worin Alk^2 eine verzweigte oder unverzweigte (C_1-C_{10})Alkylenkette darstellt und Z^1 für H oder (C_1-C_5)-Alkyl steht.

23. Verbindung gemäß Anspruch 20, worin Alk^2 und Sp^2 zusammen eine Bindung darstellen und Z^1 für H oder (C_1-C_5)-Alkyl steht.

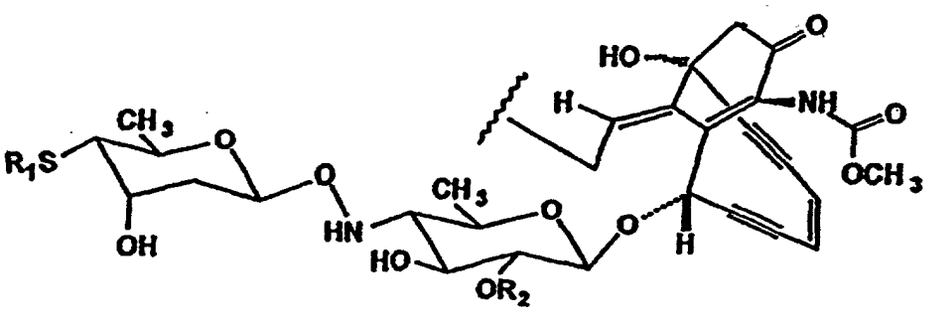
24. Verbindung gemäß Anspruch 20, worin Alk^2 und Sp^2 zusammen eine Bindung darstellen und Z^1 für Phenyl steht, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von (C_1-C_5)-Alkyl, (C_1-C_4)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$.

25. Verbindung gemäß Anspruch 20, worin Sp^1 eine Bindung, -S-, -O-, -CONH-, -NHCO- oder -NR' darstellt, unter der Voraussetzung, dass wenn Alk^1 eine Bindung darstellt, Sp^1 eine Bindung darstellt.

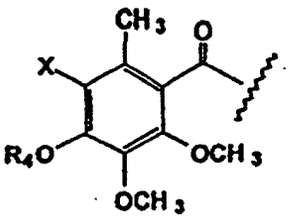
26. Verbindung gemäß Anspruch 25, worin Ar für 1,2-, 1,3- oder 1,4-Phenylen steht, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von (C_1-C_6)-Alkyl, (C_1-C_5)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro oder COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$ oder ein 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6- oder 2,7-Naphthyliden steht, jedes gegebenenfalls substituiert mit einer, zwei, drei oder vier Gruppen von (C_1-C_6)-Alkyl, (C_1-C_5)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro oder COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$.

27. Verbindung gemäß Anspruch 26, worin

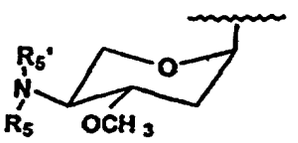
Z^2 für Q-Sp-SS-W steht, worin W für



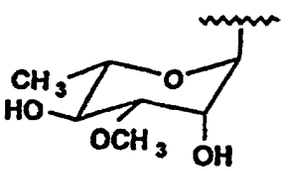
steht,
R_1



darstellt;
R_2



oder H darstellt;
R_4



oder H darstellt,
R_5, X, R_5', R und Sp wie in Anspruch 19 definiert sind; und Q für =NHNCO- steht.

28. Verbindung gemäß Anspruch 27, worin Alk^2 und Sp^2 zusammen eine Bindung darstellen, Ar für 1,2-, 1,3- oder 1,4-Phenylen steht, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von (C_1-C_6)-Alkyl, (C_1-C_5)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro oder COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$ und Z^1 für H oder (C_1-C_5)-Alkyl steht.

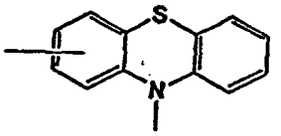
29. Verbindung gemäß Anspruch 28, worin Sp^1 für -O- steht, Alk^1 für C_4-Alkyl steht, Ar für 1,4-Phenylen steht und Z^1 für C_1-Alkyl steht.

30. Verbindung gemäß Anspruch 29, worin Z^2 Calicheamicin-gamma-dimethylhydrazid oder Calicheamicin-N-acetyl-gamma-dimethylhydrazid darstellt.

31. Verbindung der Formel:

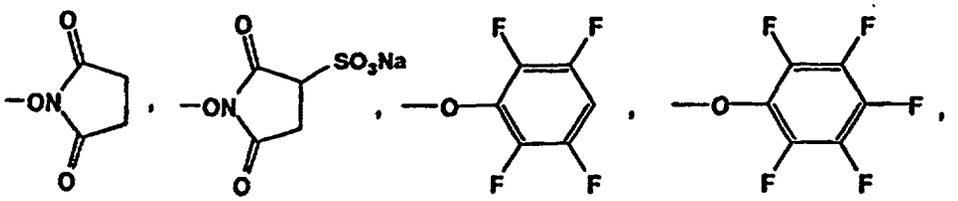
$$Z^3CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C\ (Z^1)=O,$$

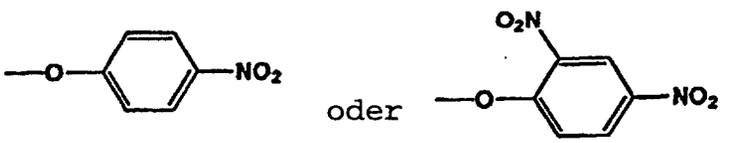
worin Ar ein 1,2-, 1,8- oder 2,7-Naphthyliden oder



darstellt, jedes Naphthyliden oder Phenothiazin gegebenenfalls substituiert mit einer, zwei, drei oder vier Gruppen von (C_1-C_6)-Alkyl, (C_1-C_5)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro oder COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$, worin n eine ganze Zahl von 0 bis 5 darstellt und R' eine verzweigte oder unverzweigte (C_1-C_5)-Kette darstellt, gegebenenfalls substituiert durch eine oder zwei Gruppen von -OH, (C_1-C_4)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro, (C_1-C_3)-Dialkylamino oder (C_1-C_3)-Trialkylammonium-A^-, worin A^- ein pharmazeutisch annehmbares Anion darstellt, welches ein Salz komplettiert, unter der Voraussetzung, dass wenn Ar Phenothiazin darstellt, Sp^1 eine nur an Stickstoff gebundene Bindung darstellt;

Z^3 Halogen, Hydroxy, OM worin M für ein Metall steht, welches ein Salz komplettiert, $-N_3$,





darstellt;
Alk^1 und Alk^2 unabhängig eine Bindung oder verzweigte oder unverzweigte (C_1-C_{10})-Alkylenkette darstellen;
Sp^1 eine Bindung, -S-, -O-, -CONH-, -NHCO-, -NR'-, $-N(CH_2CH_2)_2N-$ oder -X-Ar'-Y-$(CH_2)_n$-Z darstellt, worin X, Y und Z unabhängig eine Bindung, -NR'-, -S- oder -O- darstellen, unter der Voraussetzung, dass wenn n = 0, dann muss mindestens eins von Y und Z eine Bindung darstellen und Ar' steht für 1,2-, 1,3-oder 1,4-Phenylen, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von (C_1-C_5)-Alkyl, (C_1-C_4)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$, worin n und R' wie hierin vorher definiert sind, unter der Voraussetzung, dass wenn Alk^1 eine Bindung darstellt, Sp^1 eine Bindung darstellt;
Sp^2 eine Bindung, -S- oder -O- darstellt, unter der Voraussetzung, dass wenn Alk^2 eine Bindung darstellt, Sp^2 eine Bindung darstellt; und

Z^1 für H, (C_1-C_5)-Alkyl steht oder Phenyl, gegebenenfalls substituiert mit ein, zwei oder drei Gruppen von (C_1-C_5)-Alkyl, (C_1-C_4)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$ steht,

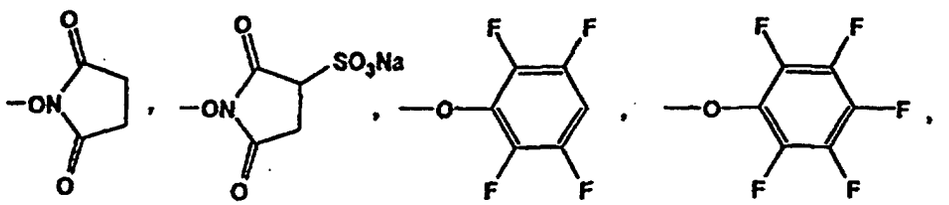
worin n und R' wie hierin vorher definiert sind, unter der Voraussetzung, dass wenn Ar für Naphthyliden steht, Z^1 nicht Wasserstoff darstellt und unter der Voraussetzung, dass wenn Alk^1 eine Bindung darstellt, Sp^2 eine Bindung darstellt und Alk^2 keine Bindung darstellt, dann Z^1 nicht C_1-Alkyl darstellt.

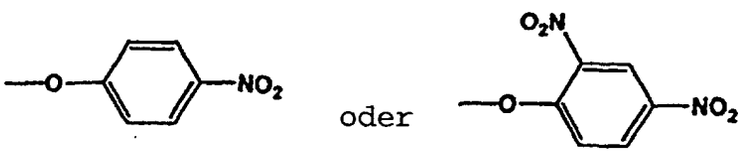
32. Verbindung der Formel:

$$Z^3CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=O$$

worin Ar 1,2-, 1,3- oder 1,4-Phenylen darstellt, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von (C_1-C_6)-Alkyl, (C_1-C_5)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro oder COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$, worin n eine ganze Zahl von 0 bis 5 darstellt und R' eine verzweigte oder unverzweigte (C_1-C_5)-Kette darstellt, gegebenenfalls substituiert durch eine oder zwei Gruppen von -OH, (C_1-C_4)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro, (C_1-C_3)-Dialkylamino oder (C_1-C_3)-Trialkylammonium-A^-, worin A^- ein pharmazeutisch annehmbares Anion darstellt, welches ein Salz komplettiert;

Z^3 Halogen, Hydroxy, OM worin M für ein Metall steht, welches ein Salz komplettiert, $-N_3$,





darstellt;

Alk^1 und Alk^2 unabhängig eine verzweigte oder unverzweigte (C_1-C_{10})-Alkylenkette darstellen;

Sp^1 eine Bindung, -S-, -O-, -CONH-, -NHCO-, -NR'-, $-N(CH_2CH_2)_2N-$ oder -X-Ar'-Y-$(CH_2)_n$-Z darstellt, worin X, Y und Z unabhängig eine Bindung, -NR'-, -S- oder -O- darstellen, unter der Voraussetzung, dass wenn n = 0, dann muss mindestens eins von Y und Z eine Bindung darstellen und Ar' steht für 1,2-, 1,3-oder 1,4-Phenylen, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von (C_1-C_5)-Alkyl, (C_1-C_4)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$, worin n und R' wie hierin vorher definiert sind;

Sp^2 eine Bindung, -S- oder -O- darstellt, unter der Voraussetzung, dass Sp^1 und Sp^2 nicht gleichzeitig eine Bindung darstellen; und

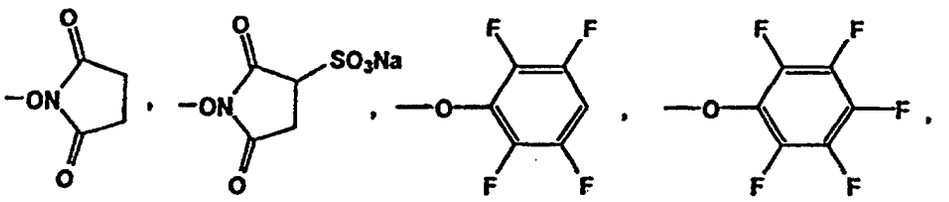
Z^1 für Phenyl steht, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von (C_1-C_5)-Alkyl, (C_1-C_4)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$ steht, worin n und R' wie hierin vorher definiert sind.

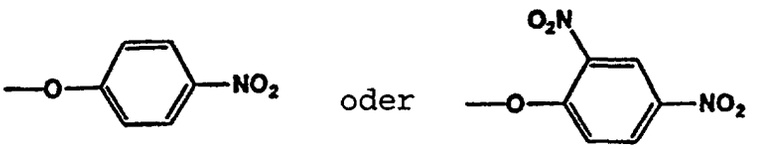
33. Verbindung der Formel:

$Z^3CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=O$,

worin Ar 1,2-, 1,3- oder 1,4-Phenylen darstellt, gegebenenfalls substituiert mit COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$ und gegebenenfalls substituiert mit einer oder zwei Gruppen von (C_1-C_6)-Alkyl, (C_1-C_5)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen oder Nitro, worin n eine ganze Zahl 0 bis 5 darstellt und R' eine verzweigte oder unverzweigte (C_1-C_5)-Kette darstellt, gegebenenfalls substituiert durch eine oder zwei Gruppen von -OH, (C_1-C_4)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro, (C_1-C_3)-Dialkylamino oder (C_1-C_3)-Trialkylammonium-A^-, worin A^- ein pharmazeutisch annehmbares Anion darstellt, welches ein Salz komplettiert;

Z^3 Halogen, Hydroxy, OM worin M für ein Metall steht, welches ein Salz komplettiert, $-N_3$,





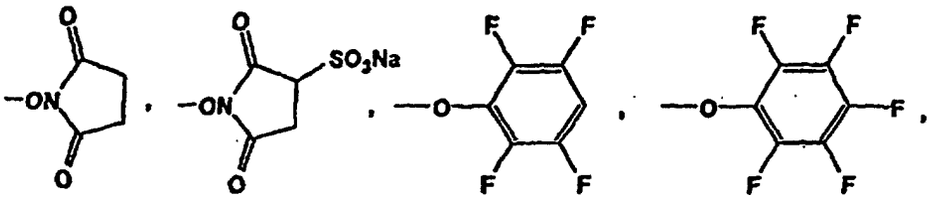
darstellt;
Alk^1 und Alk^2 unabhängig eine Bindung oder verzweigte oder unverzweigte (C_1-C_{10})-Alkylenkette darstellen;
Sp^1 eine Bindung, -S-, -O-, -CONH-, -NHCO-, -NR'-, $-N(CH_2CH_2)_2N$- oder -X-Ar'-Y-$(CH_2)_n$-Z darstellt, worin X, Y und Z unabhängig eine Bindung, -NR'-, -S- oder -O- darstellen, unter der Voraussetzung, dass wenn n = 0, dann muss mindestens eins von Y und Z eine Bindung darstellen und Ar' steht für 1,2-, 1,3-oder 1,4-Phenylen, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von (C_1-C_5)-Alkyl, (C_1-C_4)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$, unter der Voraussetzung, dass wenn Alk^1 eine Bindung darstellt, Sp^1 eine Bindung darstellt, worin n und R' wie hierin vorher in (C) definiert sind, unter der Voraussetzung, dass wenn Ar für 1,3- oder 1,4-Phenylen steht, gegebenenfalls substituiert mit einer Gruppe von (C_1-C_6)-Alkyl oder (C_1-C_5)-Alkoxy und Alk^2 eine Bindung darstellt, Sp^1 keine Bindung, -O- oder NHCO- darstellt;
Sp^2 eine Bindung, -S- oder -O- darstellt, unter der Voraussetzung, dass wenn Alk^2 eine Bindung darstellt, Sp^2 eine Bindung darstellt; und
Z^1 für H oder (C_1-C_5)-Alkyl steht.

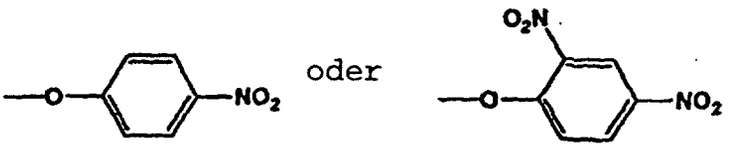
34. Verbindung der Formel:

$Z^3CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C\ (Z^1)=O$

worin Ar 1,2-, 1,3- oder 1,4-Phenylen darstellt, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von (C_1-C_6)-Alkyl, (C_1-C_5)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen oder Nitro;

Z^3 Halogen, Hydroxy, OM worin M für ein Metall steht, welches ein Salz komplettiert, $-N_3$,





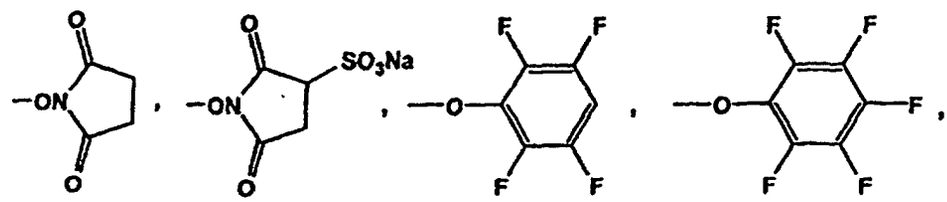
darstellt;
Alk^1 eine verzweigte oder unverzweigte (C_1-C_{10})-Alkylenkette darstellt;
Alk^2 eine Bindung oder verzweigte oder unverzweigte (C_1-C_{10})-Alkylenkette darstellt;
Sp^1 -CONH- darstellt;
Sp^2 eine Bindung, -S- oder -O- darstellt, unter der Voraussetzung, dass wenn Alk^2 eine Bindung darstellt, Sp^2 eine Bindung darstellt; und
Z^1 für H oder (C_1-C_5)-Alkyl steht;

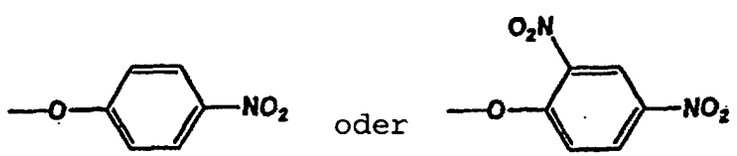
unter der Voraussetzung, dass wenn Alk^1 für C_2 steht, Alk^2 eine Bindung darstellt und Z^1 für C_1 steht, dann hat Ar mindestens einen Substituenten, unter der Voraussetzung, dass wenn Alk^1 für C_2 steht, Z^1 für H steht, Alk^2 eine Bindung darstellt und Ar für 1,4-Phenylen steht, dann hat Ar mindestens einen Substituenten, und unter der Voraussetzung, dass wenn Alk^1 für C_3 steht, Z^1 für C_1 steht, Alk^2 eine Bindung darstellt und Ar für 1,4-Phenylen steht, dann hat Ar mindestens einen Substituenten.

35. Verbindung der Formel:

$$Z^3CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=O$$

worin Ar 1,2-, 1,3- oder 1,4-Phenylen darstellt, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von (C_1-C_6)-Alkyl, (C_1-C_5)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen oder Nitro;
Z^3 Halogen, Hydroxy, OM worin M für ein Metall steht, welches ein Salz komplettiert, $-N_3$,



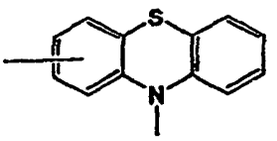


darstellt;
Alk^1 eine verzweigte oder unverzweigte (C_1-C_{10})-Alkylenkette darstellt;
Alk^2 eine Bindung oder verzweigte oder unverzweigte (C_1-C_{10})-Alkylenkette darstellt;
Sp^1 eine Bindung, -S-, $-N(CH_2CH_2)_2N-$ oder -X-Ar'-Y-$(CH_2)_n$-Z darstellt, worin X, Y und Z unabhängig eine Bindung, -NR'-, -Soder -O- darstellen, unter der Voraussetzung, dass wenn n = 0, dann muss mindestens eins von Y und Z eine Bindung darstellen und Ar' steht für 1,2-, 1,3- oder 1,4-Phenylen, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von (C_1-C_5)-Alkyl, (C_1-C_4)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$, worin n und R' wie hierin vorher definiert sind;
Sp^2 eine Bindung, -S- oder -O- darstellt, unter der Voraussetzung, dass wenn Alk^2 eine Bindung darstellt, Sp^2 eine Bindung darstellt; und
Z^1 für H oder (C_1-C_5)-Alkyl steht.

36. Verbindung gemäß Anspruch 31, worin Z^1 für H oder (C_1-C_5)-Alkyl steht und Sp^2 und Alk^2 jeweils eine Bindung darstellen.

37. Verbindung gemäß Anspruch 36, worin Sp^1 eine Bindung, -S-, -O-, -CONH-, -NHCO-, -NR'- oder $-N(CH_2CH_2)_2N-$ darstellt und Alk^1 eine verzweigte oder unverzweigte (C_1-C_{10})-Alkylenkette darstellt.

38. Verbindung gemäß Anspruch 37, worin Ar ein nicht substituiertes 1,2-, 1,8- oder 2,7-Naphthyliden oder



darstellt.

39. Verbindung gemäß Anspruch 32, worin Sp^1 eine Bindung, -S-, -O-, -CONH-, -NHCO-, -NR'- oder $-N(CH_2CH_2)_2N-$ darstellt.

40. Verbindung gemäß Ansprüchen 33, 34 oder 35, worin Alk^2 und Sp^2 jeweils eine Bindung darstellen.

41. Verbindung gemäß Anspruch 33, worin Alk^1 eine verzweigte oder unverzweigte (C_1-C_{10})-Alkylenkette darstellt und Sp^1 eine Bindung, -S-, -O-, -CONH-, -NHCO-, -NR'-, $-N(CH_2CH_2)_2N-$ darstellt und Alk^2 und Sp^2 jeweils eine Bindung darstellen.

42. Verbindung gemäß Anspruch 39, worin Ar für nicht substituiertes 1,2-, 1,3- oder 1,4-Phenylen steht und Z^1 nicht substituiertes Phenyl darstellt.

43. Verbindung gemäß Anspruch 41, worin Ar für nicht substituiertes 1,2-, 1,3- oder 1,4-Phenylen steht und Z^1 (C_1-C_5) -Alkyl darstellt.

44. Verbindung gemäß Anspruch 34, worin Alk^2 und Sp^2 jeweils eine Bindung darstellen, Ar für nicht substituiertes 1,2- 1,3 oder 1,4-Phenylen steht und Z^1 (C_1-C_5)-Alkyl darstellt.

45. Verbindung gemäß Anspruch 35, worin Sp^1 für ein -S- oder - $N(CH_2CH_2)_2N-$ steht und Alk^2 und Sp^2 jeweils eine Bindung darstellen.

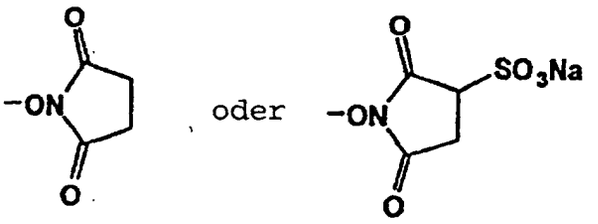
46. Verbindung gemäß den Ansprüchen 39, 41 oder 45, worin Ar für nicht substituiertes 1,2-, 1,3- oder 1,4-Phenylen steht.

47. Verbindung gemäß Anspruch 45, worin Ar für nicht substituiertes 1,2-, 1,3- oder 1,4-Phenylen steht und Z^1 (C_1-C_5) -Alkyl darstellt.

48. Verbindung gemäß Anspruch 34, worin Ar für nicht substituiertes 1,2-, 1,3- oder 1,4-Phenylen steht und Alk^2 und

Sp^2 jeweils eine Bindung darstellen.

49. Verbindung gemäß den Ansprüchen 38, 42, 43, 44 oder 47, worin Z^3 Hydroxy,



darstellt.

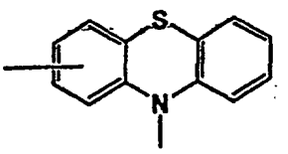
50. Pharmazeutische Zusammensetzung zum Hemmen des Wachstums von Zellen, welche eine wirksame, Zellwachstum hemmende Menge des Konjugats von Anspruch 1 und ein parenteral verabreichbares Medium umfasst.

51. Pharmazeutische Zusammensetzung zum Hemmen des Wachstums von Zellen, welche eine wirksame Zellwachstum hemmende Menge des Konjugats von Anspruch 15 oder 16 und parenteral verabreichbares Medium umfasst.

52. Gefriergetrocknete pharmazeutische Zusammensetzung zum Hemmen des Wachstums von Zellen, welche ein Konjugat von Anspruch 15 oder 16 umfasst, welche durch Gefriertrocknen einer etwa 1 mg/ml Lösung des Konjugats, gelöst in etwa 5 mM Natriumphosphat-Puffer bei einem pH von etwa 7,4, enthaltend etwa 100 mM Natriumchlorid und etwa 100 mM Sucrose, erhalten wird.

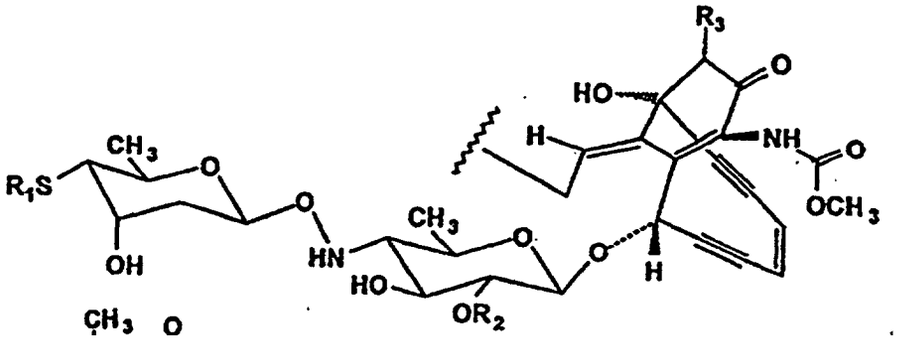
53. Verfahren zum Herstellen der anvisierten Derivate der Formel $Z^3(CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=Z^2)m$, worin

Z^3 ein Protein darstellt, ausgewählt aus mono- und polyklonalen Antikörpern, ihren Antigen-erkennenden Fragmenten und ihren chemisch oder genetisch manipulierten Gegenstücken;
Alk^1 und Alk^2 unabhängig eine Bindung oder verzweigte oder unverzweigte (C_1-C_{10})-Alkylenkette darstellen;
Sp^1 eine Bindung, -S-, -O-, -CONH-, -NHCO-, -NR'-, $-N(CH_2CH_2)_2N$- oder -X-Ar'-Y-$(CH_2)_n$-Z darstellt, worin X, Y und Z unabhängig eine Bindung, -NR'-, -S- oder -O- darstellen, unter der Voraussetzung, dass wenn n = 0, dann muss mindestens eins von Y und Z eine Bindung darstellen und Ar' steht für 1,2-, 1,3-oder 1,4-Phenylen, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von (C_1-C_5)-Alkyl, (C_1-C_4)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$, unter der Voraussetzung, dass wenn Alk^1 eine Bindung darstellt, Sp^1 eine Bindung darstellt;
n eine Ganze Zahl von 0 bis 5 darstellt;
R' eine verzweigte oder unverzweigte (C_1-C_5)-Kette darstellt, gegebenenfalls substituiert durch eine oder zwei Gruppen von -OH, (C_1-C_4)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro, (C_1-C_3)-Dialkylamino oder (C_1-C_3)-Trialkylammonium $-A^-$, worin A^- ein pharmazeutisch annehmbares Anion darstellt, welches ein Salz komplettiert;
Ar für 1,2-, 1,3- oder 1,4-Phenylen steht, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von (C_1-C_6)-Alkyl, (C_1-C_5)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro oder COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$, worin n und R' wie oben definiert sind, oder ein 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6- oder 2,7-Naphthyliden oder

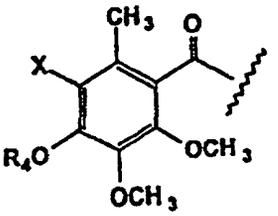


jedes Naphthyliden oder Phenothiazin gegebenenfalls substituiert mit einer, zwei, drei oder vier Gruppen von (C_1-C_6)-Alkyl, (C_1-C_5)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$, worin n und R' wie oben definiert sind, unter der Voraussetzung, dass wenn Ar für Naphthyliden steht, Z^1 nicht für Wasserstoff steht, und unter der Voraussetzung,

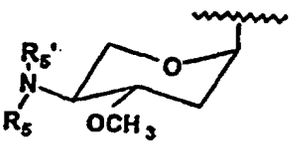
dass wenn Ar für Phenothiazin steht, Sp^1 eine nur mit Stickstoff verbundene Bindung darstellt;
Sp^2 eine Bindung, -S- oder -O- darstellt, unter der Voraussetzung, dass wenn Alk^2 eine Bindung darstellt, Sp^2 eine Bindung darstellt;
Z^1 für H, $(C_1\text{-}C_5)$-Alkyl oder Phenyl steht, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von $(C_1\text{-}C_5)$-Alkyl, $(C_1\text{-}C_4)$-Alkoxy, $(C_1\text{-}C_4)$-Thioalkoxy, Halogen, Nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$ steht, worin n und R' wie oben definiert sind;
Z^2 für Q-Sp-S-S-W steht, worin W für



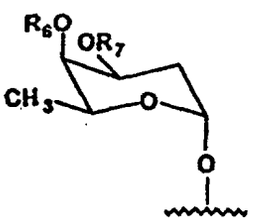
steht,
R_1



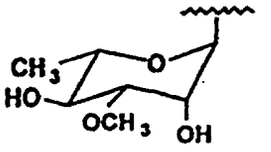
oder CH_3 darstellt;
R_2



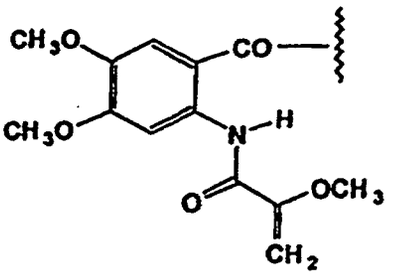
oder H darstellt;
R_3



oder H darstellt;
R_4



oder H darstellt,
R_6 oder R_7 für H oder

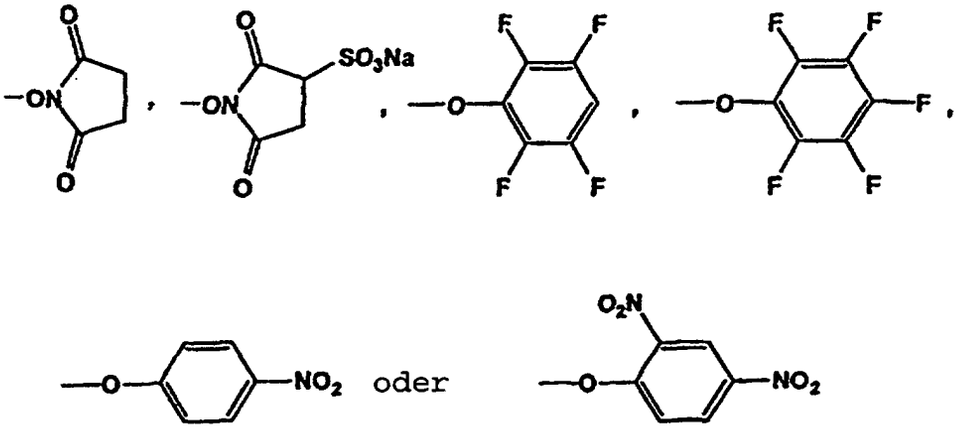


stehen;
R_5 für $-CH_3$, $-C_2H_5$ oder $-CH(CH_3)_2$ steht; X ein Iod- oder Bromatom darstellt; R_5' ein Wasserstoff oder die Gruppe RCO darstellt, worin R für Wasserstoff, verzweigtes oder unverzweigtes (C_1-C_{10})-Alkyl oder (C_1-C_{10})-Alkylengruppe, eine (C_6-C_{11})-Arylgruppe, eine (C_6-C_{11})-Arylalkyl(C_1-C_5)-gruppe oder eine Heteroaryl- oder Heteroaryl-alkyl-(C_1-C_5)-gruppe steht, worin Heteroaryl für 2- oder 3-Furyl, 2- oder 3-Thienyl, 2- oder 3-(N-Methylpyrrolyl), 2-, 3- oder 4-Pyridyl, 2-, 4- oder 5-(N-Methylimidazolyl), 2-, 4- oder 5-Oxazolyl, 2-, 3-, 5- oder 6-Pyrimidinyl, 2-, 3-, 4-, 5-, 6-, 7- oder 8-Chinolyl oder 1-, 3-, 4-, 5-, 6-, 7- oder 8-Isochinolyl steht, alles Aryl und Heteroaryl gegebenenfalls substituiert durch eine oder mehrere Hydroxy-, Amino-, Carboxy-, Halogen-, Nitro-, nied.-(C_1-C_3)-Alkoxy oder nied.-(C_1-C_5)-Thioalkoxygruppen;
Sp einen grad- oder verzweigtkettigen bivalenten oder trivalenten (C_1-C_{18})-Rest, bivalenten oder trivalenten Aryl- oder Heteroarylrest, bivalenten oder trivalenten (C_3-C_{18})-Cycloalkyloder Heterocycloalkylrest, bivalenten oder trivalenten Aryloder Heteroaryl-alkyl-(C_1-C_{18})-rest, bivalenten oder trivalenten Cycloalkyl- oder Heterocycloalkyl-alkyl-(C_1-C_{18})-rest oder bivalenten oder trivalenten (C_2-C_{18}) ungesättigten Alkylrest darstellt, worin Heteroaryl für Furyl, Thienyl, N-Methylpyrrolyl, Pyridinyl, N-Methylimidazolyl, Oxazolyl, Pyrimidinyl, Chinolyl, Isochinolyl, N-Methylcarbazoyl, Aminocumarinyl oder Phenazinyl steht und worin, wenn Sp einen trivalenten Rest darstellt, Sp zusätzlich substituiert sein kann durch nied.-(C_1-C_5)-Dialkylamino-, nied. - (C_1-C_5)-Alkoxy-, Hydroxy- oder nied-(C_1-C_5)-Alkylthiogruppen;
Q für =NHNCO-, =NHNCS-, =NHNCONH-, =NHNCSNH- oder =N0-steht; und
m von etwa 0,1 bis 15 ist;

welches umfasst: Umsetzen einer Verbindung der Formel

$$Z^{3'}CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=Z^2,$$

worin Alk^1, Sp^1, Ar, Sp^2, Alk^2, Z^1 und Z^2 wie oben definiert sind; und $Z^{3'}$ für



steht; mit einem Träger Z^3, worin Z^3 ein Protein darstellt, ausgewählt aus mono- und polyklonalen Antikörpern, ihren Antigen-erkennenden Fragmenten und ihren chemisch oder genetisch manipulierten Gegenstücken, in einer wässerigen, gepufferten Lösung bei einem pH von zwischen 6,5 und 9,0 und einer Temperatur von 4° bis 40°C für 1 bis 48 Stunden, um die anvisierten, oben definierten Derivate der Formel

$$Z^3\ (CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C\ (Z^1)\ =Z^2)m$$

zu erzeugten.

54. Verfahren, wie in Anspruch 53 beansprucht, worin die Verbindung der Formel

$$Z^{3'}CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C\ (Z^1)\ =Z^2$$

erzeugt wird durch:

(a) Umsetzen von H_2Z^2 mit einer Verbindung der Formel

$$HOCO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=0,$$

in einem alkoholischen Lösungsmittel mit einem Siedepunkt von weniger als etwa 100°C in Gegenwart von etwa 5% Essigsäure oder einem Carbonsäurekatalysator bei etwa 20° bis 70°C für etwa 1 bis 24 Stunden, worin Alk^1 und Alk^2, Sp^1, n, R', Sp^2, Z^1 und Ar wie in Anspruch 53 definiert sind, um einen Zwischenstoff der Formel

$$HOCO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=Z^2$$

herzustellen, worin Alk^1, Sp^1, Ar, Sp^2, Alk^2, Z^1 und Z^2 wie in Anspruch 53 definiert sind;
(b) Isolieren des Zwischenstoffs von Schritt (a); und (c) Umsetzen des isolierten Zwischenstoffs von Schritt (b) mit N-Hydroxysuccinimid, 2,3,5,6-Tetrafluorphenol, Pentafluorphenol, 4-Nitrophenol, 2,4-Dinitrophenol oder N-Hydroxysulfosuccinimid in Gegenwart von DCC, EDCI oder anderem Carbodiimid in einem inerten organischen Lösungsmittel wie Acetonitril oder Acetonitril, welches 5-50% DMF enthält.

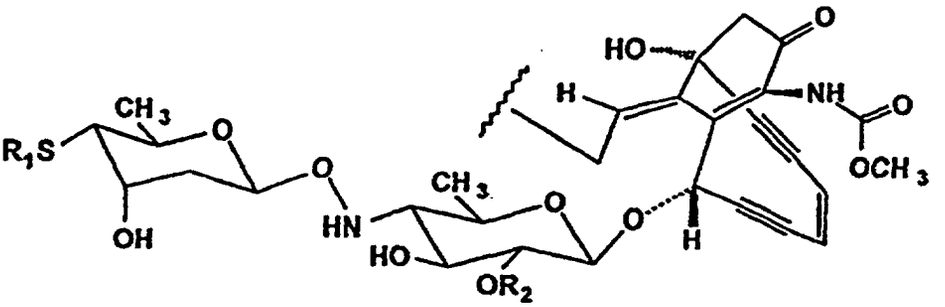
55. Verfahren nach Anspruch 53 oder Anspruch 54, worin Alk^2 und Sp^2 zusammen eine Bindung darstellen und Z^1 für H oder (C_1-C_5)-Alkyl steht.

56. Verfahren nach Anspruch 54 oder Anspruch 55, worin Sp^1 eine Bindung, -S-, -O-, -CONH-, -NHCO- oder -NR' darstellt, unter der Voraussetzung, dass wenn Sp^1 eine Bindung darstellt, Alk^1 eine Bindung darstellt.

57. Verfahren nach Anspruch 54 oder Anspruch 55, worin Ar für 1,2-, 1,3- oder 1,4-Phenylen steht, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von (C_1-C_6)-Alkyl, (C_1-C_5)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro oder COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$, oder ein 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6- oder 2,7-Naphthyliden, jedes gegebenenfalls substituiert mit einer, zwei, drei oder vier Gruppen von (C_1-C_6)-Alkyl, (C_1-C_5)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$.

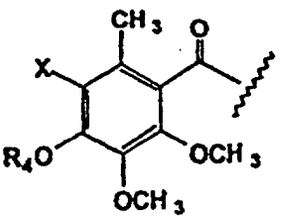
58. Verfahren nach Anspruch 54 oder Anspruch 55, wobei eine kovalente Bindung an das Z^3-Protein ein Amid ist, gebildet aus einer Umsetzung mit den Lysin-Seitenketten des Z^3-Proteins.

59. Verfahren nach Anspruch 54 oder Anspruch 55, worin Z^2 für Q-Sp-S-S-W steht und W für



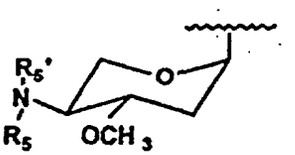
steht,

R_1



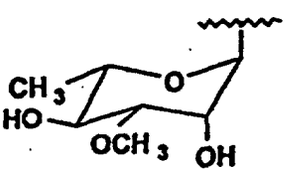
darstellt,

R_2



oder H darstellt;

R_4



oder H darstellt;
R_5, X, R_5', R und Sp wie in Anspruch 49 definiert sind und Q für =NHNCO- steht.

60. Verfahren nach Anspruch 54, wobei das alkoholische Lösungsmittel von Schritt (a) Methanol ist; der Carbonsäurekatalysator von Schritt (a) 5% Essigsäure ist; der isolierte Zwischenstoff von Schritt (b) in Schritt (c) mit N-Hydroxysuccinimid in Gegenwart von EDCI in Acetonitril umgesetzt wird; und die wässerige gepufferte Lösung von Schritt (d) Phosphatpuffer mit einem pH von 7,4 bis 8,0 ist.

61. Verfahren nach Anspruch 60, wobei Z^1 für (C_1-C_5)-Alkyl steht.

62. Verfahren nach Anspruch 61, wobei Ar für 1,2-, 1,3- oder 1,4-Phenylen steht, gegebenenfalls substituiert mit einer, zwei oder drei Gruppen von (C_1-C_6)-Alkyl, (C_1-C_5)-Alkoxy, (C_1-C_4)-Thioalkoxy, Halogen, Nitro oder COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ oder $S(CH_2)_nCONHR'$.

63. Verfahren nach Anspruch 62, wobei Sp^1 für -O- steht, Alk^1 für C_4-Alkylen steht, Ar für 1,4-Phenylen steht und Z^1 für C_1-Alkyl steht.

64. Verfahren nach Anspruch 63, wobei Z^3 für Antikörper h-P67.6, h-CT-M-01, m-CT-M-01, h-A33, m-A33 oder Anti-Tac steht.

65. Verfahren nach Anspruch 64, wobei Z^2 für Calicheamicin-gamma-dimethylhydrazid oder Calicheamicin-N-acetyl-gamma-dimethylhydrazid steht.

66. Verfahren nach Anspruch 65, wobei Z^3 für Antikörper h-CT-M-01 steht und Z^2 für Calicheamicin-N-acetyl-gamma-dimethylhydrazid steht.

67. Verfahren nach Anspruch 65, wobei Z^3 für Antikörper h-P67.6 steht und Z^2 für Calicheamicin-N-acetyl-gamma-dimethylhydrazid steht.

68. Verfahren nach Anspruch 57, wobei Sp^1 für -O- oder eine Bindung steht; Alk^1 eine Bindung oder verzweigte oder unverzweigte (C_1-C_{10})-Alkylenkette darstellt, unter der Voraussetzung, dass wenn Alk^1 eine Bindung darstellt, Sp^1 eine Bindung darstellt; Z^1 für (C_1-C_5)-Alkyl steht; und Z^2 für Calicheamicin-N-acetyl-gamma-dimethylhydrazid steht.

69. Konjugat wie in einem der Ansprüche 1 bis 18 beansprucht, zur Verwendung zur Bekämpfung des Wachstums einer unerwünschten Zelle in einem Säuger.

70. Verbindung zur Verwendung, wie in Anspruch 69 beansprucht, wobei die unerwünschte Zelle eine Krebszelle ist.

71. Verbindung zur Verwendung, wie in Anspruch 70 beansprucht, wobei der Krebs Brust-, Lungen- oder Eierstockkrebs oder Leukämie ist.

72. Verwendung eines Konjugats, wie in einem der Ansprüche 1 bis 18 beansprucht, bei der Herstellung eines Arzneimittels zur Bekämpfung des Wachstums einer unerwünschten Zelle in einem Säuger.

73. Verwendung wie in Anspruch 72 beansprucht, wobei die unerwünschte Zelle eine Krebszelle ist.

74. Verwendung wie in Anspruch 73 beansprucht, wobei der Krebs Brust-, Lungen- oder Eierstockkrebs oder Leukämie ist.

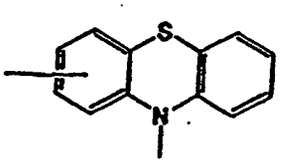
Revendications

1. Conjugué de médicament cytotoxique de formule :

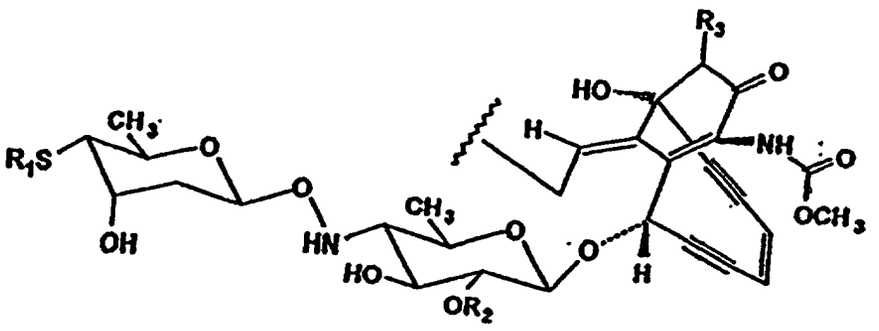
$$Z^3\ [CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=Z^2]_m$$

dans laquelle

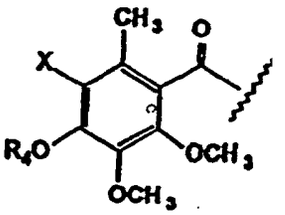
Z^3 est une protéine choisie parmi les anticorps mono- et polyclonaux, leurs fragments reconnaissant un antigène, et leurs contreparties chimiquement ou génétiquement manipulées, et les facteurs de croissance et leurs contreparties chimiquement ou génétiquement manipulées, dans laquelle une liaison covalente à la protéine est un amide formé à partir de la réaction avec des chaînes latérales de lysine «m» ou un stéroïde où la liaison covalente au stéroïde est un amide ou un ester ;
Alk^1 et Alk^2 sont indépendamment une liaison ou une chaîne alkylène ramifiée ou non ramifiée en (C_1-C_{10}) ;
Sp^1 est une liaison, -S-, -O-, -CONH-, -NHCO-, -NR'-, $-N(CH_2CH_2)_2N-$ ou -X-Ar'-Y-$(CH_2)_n$-Z où X, Y et Z sont indépendamment une liaison, -NR'-, -S- ou -O-, à condition que, quand n=0, alors au moins un des Y et Z doit être une liaison et Ar' est 1,2-, 1,3- ou 1,4-phénylène facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_5), alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, COOR', CONHR', O$(CH_2)_n$COOR', S $(CH_2)_n$COOR', O$(CH_2)_n$CONHR' ou S $(CH_2)_n$CONHR', à condition que, quand Alk^1 est une liaison, Sp^1 est aussi une liaison ;
n est un entier de 0 à 5 ;
R' est une chaîne en (C_1-C_5) ramifiée ou non ramifiée facultativement substituée par un ou deux groupements de -OH, alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, dialkylamino en (C_1-C_3) ou trialkylammonium-A^- en (C_1-C_3) où A^- est un anion pharmaceutiquement acceptable complétant un sel ;
Ar est 1,2-, 1,3- ou 1,4-phénylène, facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_6), alkoxy en (C_1-C_5), thioalkoxy en (C_1-C_4), halogène, nitro, ou COOR', CONHR', O$(CH_2)_n$COOR', S $(CH_2)_n$COOR', O$(CH_2)_n$CONHR' ou S $(CH_2)_n$CONHR' où n et R' sont comme définis ci-avant ou un 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6- ou 2,7-naphtylidène ou



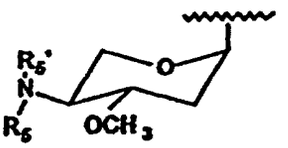
chaque naphtylidène ou phénothiazine facultativement substitué avec un, deux, trois ou quatre groupements alkyle en (C_1-C_6), alkoxy en (C_1-C_5), thioalkoxy en (C_1-C_4), halogène, nitro, COOR', CONHR', O$(CH_2)_n$COOR', S$(CH_2)_n$COOR', O$(CH_2)_n$CONHR' ou S $(CH_2)_n$CONHR' où n et R' sont comme définis ci-avant, à condition que, quand Ar est naphtylidène, Z^1 n'est pas hydrogène et à condition que quand Ar est phénothiazine, Sp^1 est une liaison uniquement connectée à l'azote ;
Sp^2 est une liaison, -S- ou -O- à condition que quand Alk^2 est une liaison, Sp^2 est aussi une liaison ;
Z^1 est H, alkyle en (C_1-C_5) ou phényle facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_5), alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, COOR', CONHR', O$(CH_2)_n$COOR', S $(CH_2)_n$COOR', O$(CH_2)_n$CONHR' ou S$(CH_2)_n$CONHR' où n et R' sont comme définis ci-dessus ;
Z^2 est Q-Sp-S-S-W, où W est



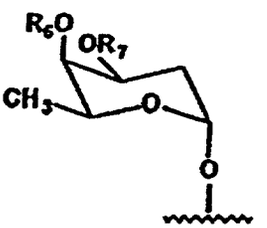
R_1 est



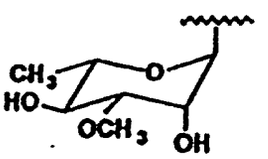
ou CH_3 ;
R_2 est



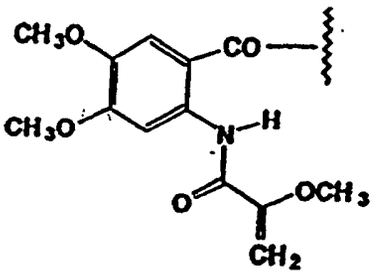
ou H ;
R_3 est



ou H ;
R^4 est



ou H ;
R_6 ou R_7 est H ou



R_5 est $-CH_3$, $-C_2H_5$ ou $-CH(CH_3)_2$; X est un atome d'iode ou de brome ; R_5' est un hydrogène ou le groupement RCO, où R est hydrogène, groupement alkyle en (C_1-C_{10}) ou alkylène en (C_1-C_{10}) ramifié ou non ramifié, un groupement aryle en (C_6-C_{11}), un groupement aryl(C_6-C_{11})-alkyle(C_1-C_5) ou un groupement hétéroaryle ou hétéroarylalkyle(C_1-C_5) dans lequel l'hétéroaryle est défini comme 2- ou 3-furyle, 2- ou 3-thiényle, 2- ou 3-(N-méthylpyrrolyle), 2-, 3- ou 4-pyridyle, 2-, 4- ou 5-(N-méthylimidazolyle), 2-, 4- ou 5-oxazolyle, 2-, 3-, 5- ou 6-pyrimidinyle, 2-, 3-, 4-, 5-, 6-, 7- ou 8-quinolyle, ou 1-, 3-, 4-, 5-, 6-, 7- ou 8-isoquinolyle, tous les groupements aryles et hétéroaryles étant facultativement substitués par un ou plusieurs groupements hydroxy, amino, carboxy, halogène, nitro, alkoxy inférieur en (C_1-C_3) ou thioalkoxy inférieur en (C_1-C_5);
Sp est un radical divalent ou trivalent à chaîne droite ou ramifiée en (C_1-C_{18}), un radical divalent ou trivalent aryle ou hétéroaryle, un radical divalent ou trivalent cycloalkyle en (C_3-C_{18}) ou hétérocycloalkyle, un radical divalent ou trivalent aryl- ou hétéroaryl-alkyle en (C_1-C_{18}), un radical divalent ou trivalent cycloalkyl- ou hétérocycloalkyl-alkyle en (C_1-C_{18}), ou un radical divalent ou trivalent alkyle insaturé en (C_2-C_{18}), où l'hétéroaryle est furyle, thiényle, N-méthylpyrrolyle, pyridinyle, N-méthylimidazolyle, oxazolyle, pyrimidinyle, quinolyle, isoquinolyle, N-méthylcarbazoyle, aminocoumarinyle ou phénazinyle et où si Sp est un radical trivalent, il peut être en plus substitué par un groupement dialkylamino inférieur en (C_1-C_5), alkoxy inférieur en (C_1-C_5), hydroxy ou alkylkthio inférieur en (C_1-C_5) ;
Q est =NHNCO-, =NHNCS-, =NHNCONH-, =NHNCSNH- ou =NHO- ; m est d'environ 0,1 à 15.

2. Conjugué de médicament cytotoxique selon la revendication 1, où Alk^2 est une chaîne alkylène en (C_1-C_{10}) ramifiée ou non ramifiée, et Z^1 est un phényle facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_5), alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ ou $S(CH_2)_nCONHR'$ où n et R' sont comme définis dans la revendication 1.

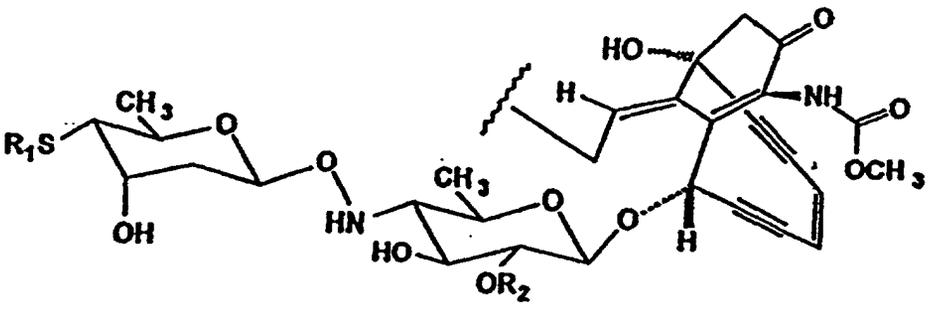
3. Conjugué de médicament cytotoxique selon la revendication 1, où Alk^2 et Sp^2 sont conjointement une liaison et Z^1 est H ou alkyle en (C_1-C_5).

4. Conjugué de médicament cytotoxique selon la revendication 1, où Sp^1 est une liaison, -S-, -O-, -CONH-, -NHCO- ou -NR' où R' est défini dans la revendication 1, à condition que, quand Alk^1 est une liaison, Sp^1 est une liaison.

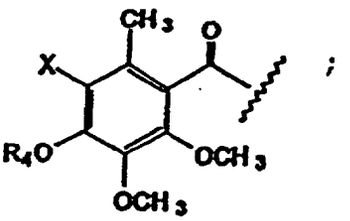
5. Conjugué de médicament cytotoxique selon la revendication 4, où Ar est 1,2-, 1,3- ou 1,4-phénylène, facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_6), alkoxy en (C_1-C_5), thioalkoxy en (C_1-C_4), halogène, nitro, ou COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ ou $S(CH_2)_nCONHR'$ où n et R' sont comme définis dans la revendication 1, ou Ar est un 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6- ou 2,7-naphtylidène, chacun facultativement substitué avec un, deux, trois ou quatre groupements alkyle en (C_1-C_6), alkoxy en (C_1-C_5), thioalkoxy en (C_1-C_4), halogène, nitro, ou COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ ou $S(CH_2)_nCONHR'$ où n et R' sont comme définis dans la revendication 1.

6. Conjugué de médicament cytotoxique selon la revendication 5, dans lequel Z^3 est une protéine choisie parmi les anticorps mono- et polyclonaux, leurs fragments reconnaissant un antigène et leurs contreparties chimiquement ou génétiquement manipulées et les facteurs de croissance et leurs contreparties chimiquement ou génétiquement manipulées, où une liaison covalente à la protéine est un amide formé par la réaction avec des chaînes latérales de lysine «m».

7. Conjugué de médicament cytotoxique selon la revendication 6, dans lequel Z^2 est Q-Sp-S-S-W où W est

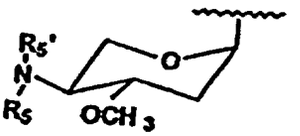


R_1 est



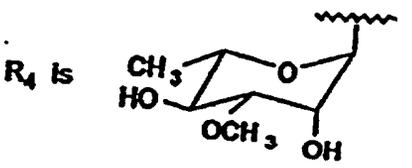
;

R_2 est



ou H ;

R_4 est



ou H ;

R_5, X, $R_{5'}$, R et Sp sont comme définis dans la revendication 1 ; et Q est =NHNCO-.

8. Conjugué de médicament cytotoxique selon la revendication 7, dans lequel Ar est 1,2-, 1,3- ou 1,4-phénylène, facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_6), alkoxy en (C_1-C_5), thioalkoxy en (C_1-C_4), halogène, nitro, ou COOR', CONHR', O $(CH_2)_n$COOR', S $(CH_2)_n$COOR', O$(CH_2)_n$CONHR' ou S

$(CH_2)_nCONHR'$ où n et R' sont comme définis dans la revendication 1, Alk^2 et Sp^2 sont conjointement une liaison, et Z^1 est H ou alkyle en (C_1-C_5).

9. Conjugué de médicament cytotoxique selon la revendication 8, dans lequel Sp^1 est -O- ou une liaison, Alk^1 est alkylène en (C_1-C_6), Ar est 1,3- ou 1,4-phénylène facultativement substitué avec un ou deux groupements alkyle en (C_1-C_3), alkoxy en (C_1-C_3), halogène, nitro, COOR' ou CONHR' où R' est comme défini dans la revendication 1, et Z^1 est alkyle en (C_1-C_3).

10. Conjugué de médicament cytotoxique selon la revendication 9, dans lequel Z^3 est un anticorps monoclonal qui reconnaît l'antigène CD33 et Z^2 est le calichéamicine gamma diméthylhydrazide ou le calichéamicine N-acétyl gamma diméthylhydrazide.

11. Conjugué de médicament cytotoxique selon la revendication 9, dans lequel Z^3 est un anticorps monoclonal qui reconnaît l'antigène de la mucine polyépithéliale et Z^2 est le calichéamicine gamma diméthylhydrazide ou le calichéamicine N-acétyl gamma diméthylhydrazide.

12. Conjugué de médicament cytotoxique selon la revendication 9, dans lequel Z^3 est un anticorps monoclonal qui reconnaît un antigène de glycoprotéine présent sur les cellules cancéreuses du colon et Z^2 est le calichéamicine gamma diméthylhydrazide ou le calichéamicine N-acétyl gamma diméthylhydrazide.

13. Conjugué de médicament cytotoxique selon la revendication 9, dans lequel Z^3 est un anticorps monoclonal qui reconnaît le récepteur IL2 trouvé sur les cellules choisies parmi le groupe constitué des cellules T activées et fonctionnellement matures et les cellules de leucémie anormalement activées et Z^2 est le calichéamicine gamma diméthylhydrazide ou le calichéamicine N-acétyl gamma diméthylhydrazide.

14. Conjugué de médicament cytotoxique selon la revendication 9, dans lequel Z^3 est l'anticorps h- ou m-P67.6, h- ou m-CT-M-01, h- ou m-A33, ou anti-Tac et Z^2 est le calichéamicine gamma diméthylhydrazide ou le calichéamicine N-acétyl gamma diméthylhydrazide.

15. Conjugué de médicament cytotoxique selon la revendication 14, dans lequel Z^3 est l'anticorps h-CT-M-01 et Z^2 est le calichéamicine N-acétyl gamma diméthylhydrazide.

16. Conjugué de médicament cytotoxique selon la revendication 14, dans lequel Z^3 est l'anticorps h-P67.6 et Z^2 est le calichéamicine N-acétyl gamma diméthylhydrazide.

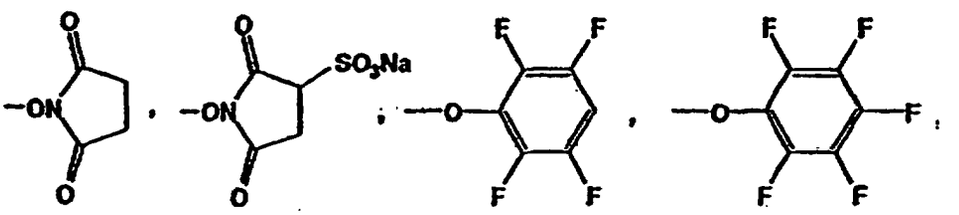
17. Conjugué de médicament cytotoxique selon la revendication 14, dans lequel Z^3 est l'anticorps h-P67.6, Z^2 est le calichéamicine N-acétyl gamma diméthylhydrazide, Sp^1 est -O-, Alk^1 est alkylène en C_3, Ar est 1,4-phénylène et Z^1 est alkyle en C_1.

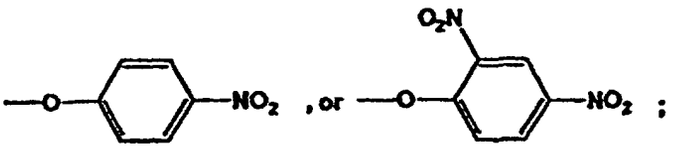
18. Conjugué de médicament cytotoxique selon la revendication 8, dans lequel Sp^1 est -O- ou une liaison, Alk^1 est une liaison ou une chaîne alkylène en (C_1-C_{10}) ramifiée ou non ramifiée, à condition que quand Alk^1 est une liaison, Sp^1 est une liaison, Z^1 est alkyle en (C_1-C_5) et Z^2 est le calichéamicine N-acétyl gamma diméthylhydrazide.

19. Composé de formule

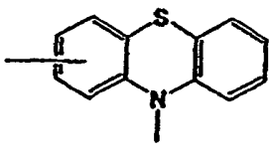
$$Z^3\ [CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=Z^2]_m$$

où Z^3 est halogène, hydroxy, OM où M est un métal complétant un sel, $-N_3$,

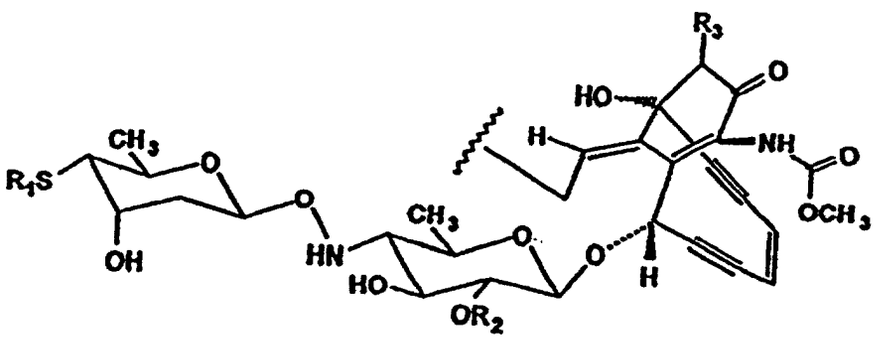




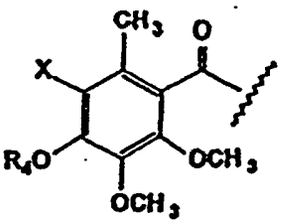
Alk^1 et Alk^2 sont indépendamment une liaison ou une chaîne alkylène ramifiée ou non ramifiée en (C_1-C_{10}) ;
Sp^1 est une liaison, -S-, -O-, -CONH-, -NHCO-, -NR'-, -N(CH_2CH_2)$_2$N- ou -X-Ar'-Y-(CH_2)$_n$-Z où X, Y et Z sont indépendamment une liaison, -NR'-; -S- ou -O-, à condition que, quand n=0, alors au moins un des Y et Z doit être une liaison et Ar' est 1,2-, 1,3- ou 1,4-phénylène facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_5), alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, COOR', CONHR', O(CH_2)$_n$COOR', S(CH_2)$_n$COOR', O(CH_2)$_n$CONHR' ou S(CH_2)$_n$CONHR', à condition que quand Alk^1 est une liaison, Sp^1 est une liaison ;
n est un entier de 0 à 5 ;
R' est une chaîne en (C_1-C_5) ramifiée ou non ramifiée facultativement substituée par un ou deux groupements de -OH, alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, dialkylamino en (C_1-C_3) ou trialkylammonium-A$^-$ en (C_1-C_3) où A$^-$ est un anion pharmaceutiquement acceptable complétant un sel ;
Ar est 1,2-, 1,3- ou 1,4-phénylène, facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_6), alkoxy en (C_1-C_5), thioalkoxy en (C_1-C_4), halogène, nitro, ou COOR', CONHR', O(CH_2)$_n$COOR', S(CH_2)$_n$COOR', O(CH_2)$_n$CONHR' ou S(CH_2)$_n$CONHR' où n et R' sont comme définis ci-avant ou un 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6- ou 2,7-naphtylidène ou



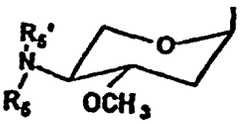
chaque naphtylidène ou phénothiazine facultativement substitué avec un, deux, trois ou quatre groupements alkyle en (C_1-C_6), alkoxy en (C_1-C_5), thioalkoxy en (C_1-C_4), halogène, nitro, ou COOR', CONHR', O(CH_2)$_n$COOR', S(CH_2)$_n$COOR', O(CH_2)$_n$CONHR' ou S (CH_2)$_n$CONHR' où n et R' sont comme définis ci-dessus, à condition que quand Ar est naphtylidène, Z^1 n'est pas hydrogène et à condition que, quand Ar est phénothiazine, Sp^1 est une liaison uniquement connectée à l'azote ;
Sp^2 est une liaison, -S- ou -O-, à condition que quand Alk^2 est une liaison, Sp^2 est une liaison ;
Z^1 est H, alkyle en (C_1-C_5) ou phényle facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_5), alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, COOR', CONHR', O(CH_2)$_n$COOR', S(CH_2)$_n$COOR', O(CH_2)$_n$CONHR' ou S(CH_2)$_n$CONHR' où n et R' sont comme définis ci-avant ;
Z^2 est Q-Sp-S-S-W, où W est



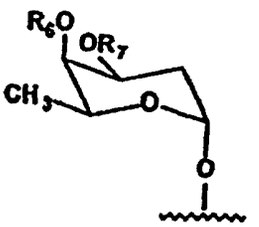
R_1 est



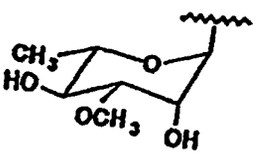
ou CH_3 ;
R_2 est



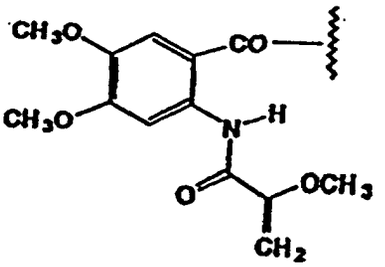
ou H ;
R_3 est



ou H ;
R^4 est

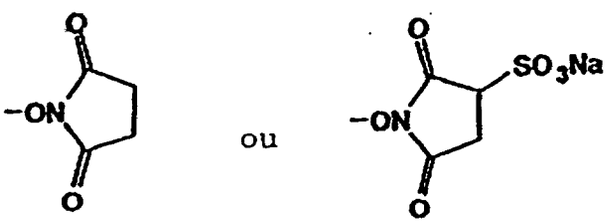


ou H ;
R_6 ou R_7 est H ou



R_5 est $-CH_3$, $-C_2H_5$ ou $-CH(CH_3)_2$; X est un atome d'iode ou de brome ; R_5' est un hydrogène ou le groupement RCO, où R est hydrogène, groupement alkyle en (C_1-C_{10}) ou alkylène en (C_1-C_{10}) ramifié ou non ramifié, un groupement aryle en (C_6-C_{11}), un groupement aryl(C_6-C_{11})-alkyle(C_1-C_5) ou un groupement hétéroaryle ou hétéroarylalkyle(C_1-C_5) dans lequel l'hétéroaryle est défini comme 2- ou 3-furyle, 2- ou 3-thiényle, 2- ou 3-(N-méthylpyrrolyle), 2-, 3- ou 4-pyridyle, 2-, 4- ou 5-(N-méthylimidazolyle), 2-, 4- ou 5-oxazolyle, 2-, 3-, 5- ou 6-pyrimidinyle, 2-, 3-, 4-, 5-, 6-, 7- ou 8-quinolyle, ou 1-, 3-, 4-, 5-, 6-, 7- ou 8-isoquinolyle, tous les groupements aryles et hétéroaryles étant facultativement substitués par un ou plusieurs groupements hydroxy, amino, carboxy, halogène, nitro, alkoxy inférieur en (C_1-C_3) ou thioalkoxy inférieur en (C_1-C_5) ;
Sp est un radical divalent ou trivalent à chaîne droite ou ramifiée en (C_1-C_{18}), un radical divalent ou trivalent aryle ou hétéroaryle, un radical divalent ou trivalent cycloalkyle en (C_3-C_{18}) ou hétérocycloalkyle, un radical divalent ou trivalent aryl- ou hétéroaryl-alkyle en (C_1-C_{18}), un radical divalent ou trivalent cycloalkyl- ou hétérocycloalkyl-alkyle en (C_1-C_{18}), ou un radical divalent ou trivalent alkyle insaturé en (C_2-C_{18}), où l'hétéroaryle est furyle, thiényle, N-méthylpyrrolyle, pyridinyle, N-méthylimidazolyle, oxazolyle, pyrimidinyle, quinolyle, isoquinolyle, N-méthylcarbazoyle, aminocoumarinyle ou phénazinyle et où si Sp est un radical trivalent, il peut être en plus substitué par un groupement dialkylamino inférieur en (C_1-C_5), alkoxy en (C_1-C_5), hydroxy ou alkylkthio inférieur en (C_1-C_5) ;
Q est =NHNCO-, =NHNCS-, =NHNCONH-, =NHNCSNH- ou =NO-.

20. Composé selon la revendication 19, dans lequel Z^3 est hydroxy,



21. Composé selon la revendication 20, dans lequel Alk^2 est une chaîne alkylène en (C_1-C_{10}) ramifiée ou non ramifiée et Z^1 est un phényle facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_5), alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ ou $S(CH_2)_nCONHR'$.

22. Composé selon la revendication 20, dans lequel Alk^2 est une chaîne alkylène en (C_1-C_{10}) ramifiée ou non ramifiée et Z^1 est H ou alkyle en (C_1-C_5).

23. Composé selon la revendication 20, dans lequel Alk^2 et Sp^2 sont conjointement une liaison et Z^1 est H ou alkyle en (C_1-C_5).

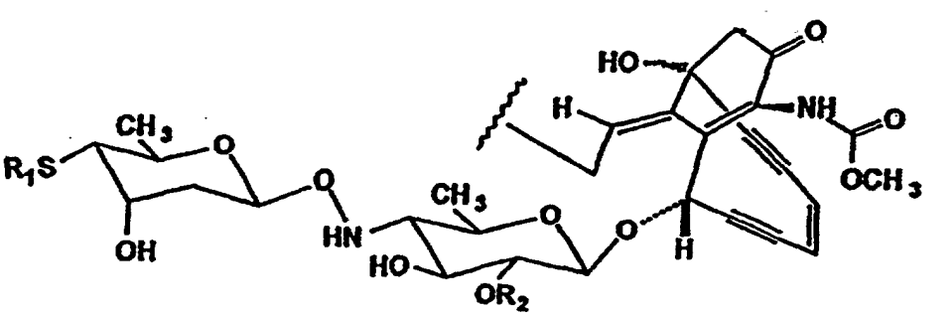
24. Composé selon la revendication 20, dans lequel Alk^2 et Sp^2 sont conjointement une liaison et Z^1 est un phényle facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_5), alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ ou $S(CH_2)_nCONHR'$.

25. Composé selon la revendication 20, dans lequel Sp^1 est une liaison, -S-, -O-, -CONH-, -NHCO- ou -NR', à condition que, quand Alk^1 est une liaison, Sp^1 est une liaison.

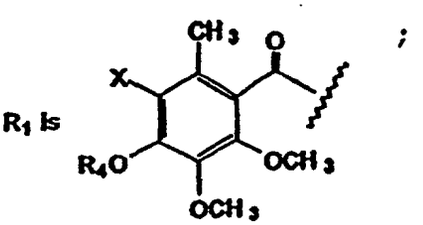
26. Composé selon la revendication 25, dans lequel Ar est 1,2-, 1,3- ou 1,4-phénylène, facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_6), alkoxy en (C_1-C_5), thioalkoxy en (C_1-C_4), halogène, nitro, ou COOR', CONHR', O $(CH_2)_n$COOR', S $(CH_2)_n$COOR', O$(CH_2)_n$CONHR' ou S $(CH_2)_n$CONHR' où n et R' sont comme définis ci-avant ou un 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6- ou 2,7-naphtylidène, chacun facultativement substitué avec un, deux, trois ou quatre groupements alkyle en (C_1-C_6), alkoxy en (C_1-C_5), thioalkoxy en (C_1-C_4), halogène, nitro, ou COOR', CONHR', O $(CH_2)_n$COOR', S$(CH_2)_n$COOR', O$(CH_2)_n$CONHR' ou S $(CH_2)_n$CONHR'.

27. Composé selon la revendication 26, dans lequel

Z^2 est Q-Sp-S-S-W, où W est

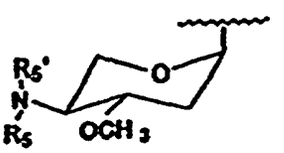


R_1 est



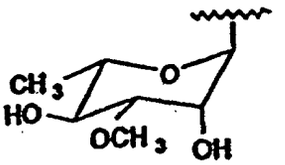
;

R_2 est



ou H ;

R_4 est



ou H ;

R_5, X, $R_{5'}$, R et Sp sont comme définis dans la revendication 19 ; et

Q est =NHNCO-.

28. Composé selon la revendication 27, dans lequel Alk^2 et Sp^2 sont conjointement une liaison, Ar est 1,2-, 1,3- ou 1,4-phénylène, facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_6), alkoxy en (C_1-C_5), thioalkoxy en (C_1-C_4), halogène, nitro, ou COOR', CONHR', O $(CH_2)_nCOOR'$, S $(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ ou $S(CH_2)_nCONHR'$, et Z^1 est H ou alkyle en (C_1-C_5).

29. Composé selon la revendication 28, dans lequel Sp^1 est -O-, Alk^1 est alkyle en C_4, Ar est 1,4-phénylène, et Z^1 est alkyle en C_1.

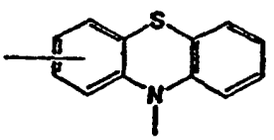
30. Composé selon la revendication 29, dans lequel Z^2 est le calichéamicine gamma diméthylhydrazide ou le calichéamicine N-acétyl gamma diméthylhydrazide.

31. Composé de formule

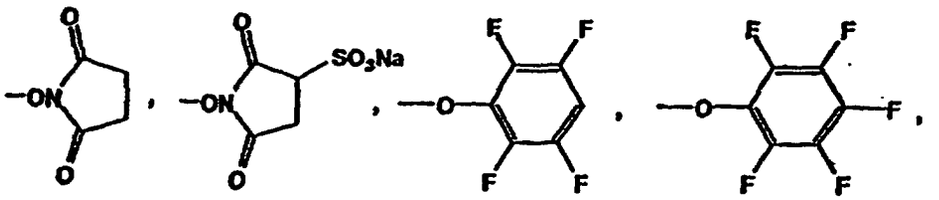
$$Z^3CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=O$$

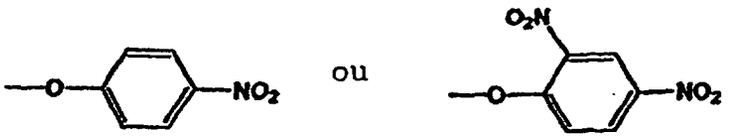
dans laquelle

Ar est 1,2-, 1,8- ou 2,7-naphtylidène ou



chaque naphtylidène ou phénothiazine facultativement substitué avec un, deux, trois ou quatre groupements alkyle en (C_1-C_6), alkoxy en (C_1-C_5), thioalkoxy en (C_1-C_4), halogène, nitro, ou COOR', CONHR', O $(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ ou $S(CH_2)_nCONHR'$ où n est un entier de 0 à 5, et R' est une chaîne en (C_1-C_5) ramifiée ou non ramifiée facultativement substituée par un ou deux groupements de -OH, alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, dialkylamino en (C_1-C_3) ou trialkylammonium-A^- en (C_1-C_3) où A^- est un anion pharmaceutiquement acceptable complétant un sel, à condition que, quand Ar est phénothiazine, Sp^1 est une liaison uniquement connectée à l'azote ;
Z^3 est halogène, hydroxy, OM où M est un métal complétant un sel, $-N_3$,





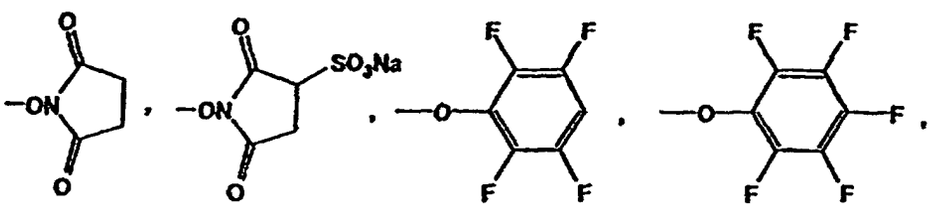
Alk^1 et Alk^2 sont indépendamment une liaison ou une chaîne alkylène ramifiée ou non ramifiée en (C_1-C_{10}) ; Sp^1 est une liaison, -S-, -O-, -CONH-, -NHCO-, -NR'-, -N(CH_2CH_2)$_2$N-, ou -X-Ar'-Y-(CH_2)$_n$-Z où X, Y et Z sont indépendamment une liaison, -NR'-, -S- ou -O-, à condition que, quand n=0, alors au moins un des Y et Z doit être une liaison et Ar' est 1,2-, 1,3- ou 1,4-phénylène facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_5), alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, COOR', CONHR', O(CH_2)$_n$COOR', S(CH_2)$_n$COOR', O(CH_2)$_n$CONHR' ou S(CH_2)$_n$CONHR', où n et R' sont comme définis avant, à condition que, quand Alk^1 est une liaison, Sp^1 est une liaison ; et
Sp^2 est une liaison, -S- ou -O-, à condition que quand Alk^2 est une liaison, Sp^2 est une liaison ;
Z^1 est H, alkyle en (C_1-C_5) ou phényle facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_5), alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, COOR', CONHR', O(CH_2)$_n$COOR', S(CH_2)$_n$COOR', O(CH_2)$_n$CONHR' ou S(CH_2)$_n$CONHR' où n et R' sont comme définis ci-avant, à condition que, quand Ar est naphtylidène, Z^1 n'est pas hydrogène et à condition que, quand Alk^1 est une liaison, Sp^2 est une liaison, et Alk^2 n'est pas une liaison, alors Z^1 n'est pas alkyle en C_1.

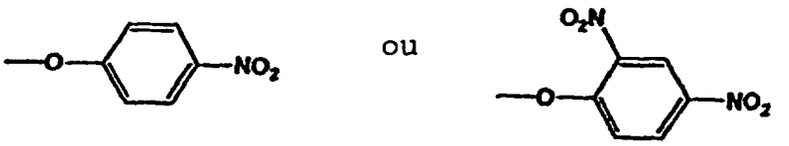
32. Composé de formule :

$$Z^3CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C\ (Z^1)=O$$

dans laquelle

Ar est 1,2-, 1,3- ou 1,4-phénylène, facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_6), alkoxy en (C_1-C_5), thioalkoxy en (C_1-C_4), halogène, nitro, ou COOR', CONHR', O(CH_2)$_n$COOR', S(CH_2)$_n$COOR', O(CH_2)$_n$CONHR' ou S(CH_2)$_n$CONHR' où n est un entier de 0 à 5, et R' est une chaîne en (C_1-C_5) ramifiée ou non ramifiée facultativement substituée par un ou deux groupements de -OH, alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, dialkylamino en (C_1-C_3) ou trialkylammonium-A$^-$ en (C_1-C_3) où A$^-$ est un anion pharmaceutiquement acceptable complétant un sel ;
Z^3 est halogène, hydroxy, OM où M est un métal complétant un sel, -N_3,





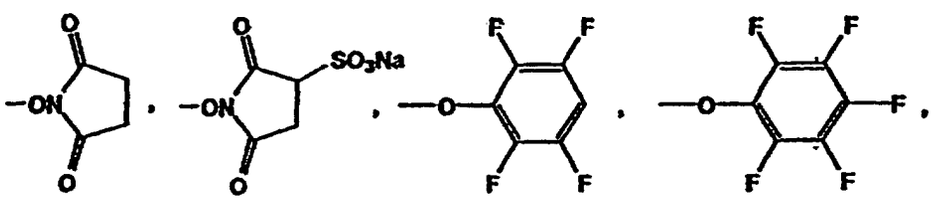
Alk^1 et Alk^2 sont indépendamment une liaison ou une chaîne alkylène ramifiée ou non ramifiée en (C_1-C_{10}) ; Sp^1 est une liaison, -S-, -O-, -CONH-, -NHCO-, -NR'-, -N(CH_2CH_2)$_2$N- ou -X-Ar'-Y-(CH_2)$_n$-Z où X, Y et Z sont indépendamment une liaison, -NR'-, -S- ou -O-, à condition que, quand n=0, alors au moins un des Y et Z doit être une liaison et Ar' est 1,2-, 1,3- ou 1,4-phénylène facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_5), alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, COOR', CONHR', O (CH_2)$_n$COOR', S (CH_2)$_n$COOR', O(CH_2)$_n$CONHR' ou S (CH_2)$_n$CONHR', où n et R' sont comme ci-avant définis ;
Sp^2 est une liaison, -S- ou -O-, à condition que Sp^1 et Sp^2 ne sont pas simultanément une liaison ; et
Z^1 est phényle facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_5), alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, COOR', CONHR', O (CH_2)$_n$COOR', S (CH_2)$_n$COOR', O (CH_2)$_n$CONHR' ou S (CH_2)$_n$CONHR' où n et R' sont comme définis ci-avant

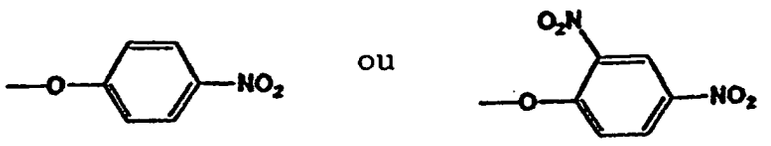
33. Composé de formule :

$$Z^3CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=O$$

dans laquelle

Ar est 1,2-, 1,3- ou 1,4-phénylène substitué avec COOR', CONHR', O (CH_2)$_n$COOR', S (CH_2)$_n$COOR', O (CH_2)$_n$CONHR' ou S(CH_2)$_n$CONHR' et facultativement substitué avec un ou deux groupements alkyle en (C_1-C_6), alkoxy en (C_1-C_5), thioalkoxy en (C_1-C_4), halogène, nitro, où n est un entier de 0 à 5, et R' est une chaîne en (C_1-C_5) ramifiée ou non ramifiée facultativement substituée par un ou deux groupements de -OH, alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, dialkylamino en (C_1-C_3) ou trialkylammonium-A$^-$ en (C_1-C_3) où A$^-$ est un anion pharmaceutiquement acceptable complétant un sel ;
Z^3 est halogène, hydroxy, OM où M est un métal complétant un sel, -N_3,





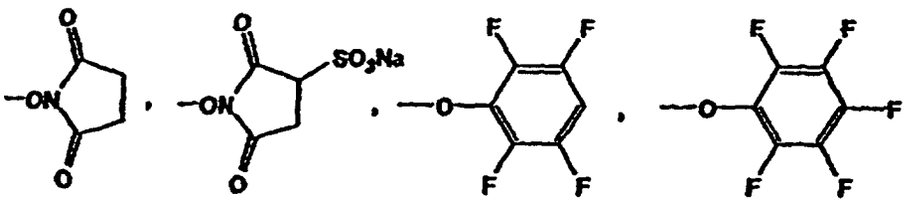
Alk^1 et Alk^2 sont indépendamment une liaison ou une chaîne alkylène ramifiée ou non ramifiée en (C_1-C_{10}) ;
Sp^1 est une liaison, -S-, -O-, -CONH-, -NHCO-, -NR'-, -$N(CH_2CH_2)_2N$- ou -X-Ar'-Y-$(CH_2)_n$-Z où X, Y et Z sont indépendamment une liaison, -NR'-, -S- ou -O-, à condition que quand n=0, alors au moins un des Y et Z doit être une liaison et Ar' est 1,2-, 1,3- ou 1,4-phénylène facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_5), alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, COOR', CONHR', O$(CH_2)_n$COOR', S $(CH_2)_n$COOR', O$(CH_2)_n$CONHR' ou S $(CH_2)_n$CONHR', à condition que quand Alk^1 est une liaison, Sp^1 est une liaison, où n et R' sont comme définis ci-avant en (C), à condition que, quand Ar est 1,3- ou 1,4-phénylène facultativement substitué avec un groupement alkyle en (C_1-C_6) ou alkoxy en (C_1-C_5) et Alk^2 est une liaison, alors Sp^1 n'est pas une liaison, -O- ou -NHCO- ;
Sp^2 est une liaison, -S- ou -O-, à condition que quand Alk^2 est une liaison, Sp^2 est une liaison ; et
Z^1 est H ou alkyle en (C_1-C_5).

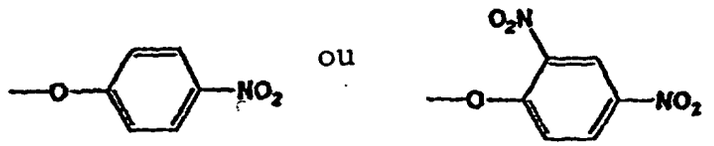
34. Composé de formule :

$$Z^3CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=O$$

dans laquelle

Ar est 1,2-, 1,3- ou 1,4-phénylène facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_6), alkoxy en (C_1-C_5), thioalkoxy en (C_1-C_4), halogène ou nitro ;
Z^3 est halogène, hydroxy, OM où M est un métal complétant un sel, -N_3,





Alk^1 est une chaîne alkylène ramifiée ou non ramifiée en (C_1-C_{10}) ;
Alk^2 est une liaison ou une chaîne alkylène ramifiée ou non ramifiée en (C_1-C_{10}) ;
Sp^1 est -CONH- ;
Sp^2 est une liaison, -S- ou -O- à condition que, quand Alk^2 est une liaison, Sp^2 est une liaison ; et
Z^1 est H ou alkyle en (C_1-C_5) ;

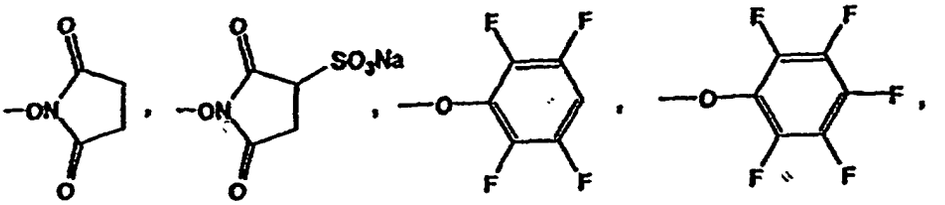
à condition que quand Alk^1 est C_2, Alk^2 est une liaison, et Z^1 est C_1, alors Ar a au moins un substituant, à condition que quand Alk^1 est C_2, Z^1 est H, Alk^2 est une liaison, et Ar est 1,4-phénylène, alors Ar a au moins un substituant et à condition que, quand Alk^1 est C_3, Z^1 est C_1, Alk^2 est une liaison et Ar est 1,4-phnéylène, alors Ar a au moins un substituant.

35. Composé de formule :

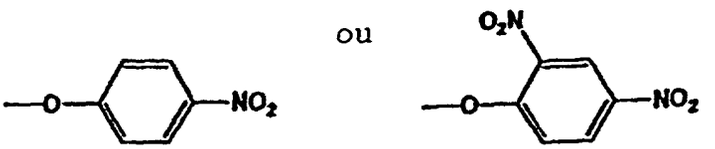
$$Z^3CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C\,(Z^1)\,=O$$

dans laquelle

Ar est 1,2-, 1,3- ou 1,4-phénylène facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_6), alkoxy en (C_1-C_5), thioalkoxy en (C_1-C_4), halogène ou nitro ;
Z^3 est halogène, hydroxy, OM où M est un métal complétant un sel, $-N_3$,



ou



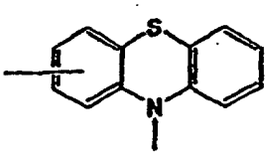
Alk^1 est une chaîne alkylène ramifiée ou non ramifiée en (C_1-C_{10}) ;
Alk^2 est une liaison ou une chaîne alkylène ramifiée ou non ramifiée en (C_1-C_{10}) ;
Sp^1 est -S-, $-N(CH_2CH_2)_2N-$ ou -X-Ar'-Y-$(CH_2)_n$-Z où X, Y et Z sont indépendamment une liaison, -NR'-, -S- ou -O-, à condition que, quand n=0, alors au moins un des Y et Z doit être une liaison et Ar' est 1,2-, 1,3- ou 1,4-phénylène facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_5), alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ ou $S(CH_2)_nCONHR'$, où n et R' sont comme définis ci-avant;
Sp^2 est une liaison, -S- ou -O- à condition que, quand Alk^2 est une liaison, Sp^2 est une liaison ; et
Z^1 est H ou alkyle en (C_1-C_5).

36. Composé selon la revendication 31, dans lequel Z^1 est H ou alkyle en (C_1-C_5) et Sp^2 et Alk^2 sont chacun une liaison.

37. Composé selon la revendication 36, dans lequel Sp^1 est une liaison, -S-, -O-, -CONH-, -NHCO-, -NR'- ou $-N(CH_2CH_2)_2N-$, et Alk^1 est une chaîne alkylène ramifiée ou non ramifiée en (C_1-C_{10}).

38. Composé selon la revendication 37, dans lequel

Ar est un 1,2-, 1,8- ou 2,7-naphtylidène non substitué ou



39. Composé selon la revendication 32, dans lequel Sp^1 est une liaison, -S-, -O-, -CONH-, -NHCO-, -NR'- ou $-N(CH_2CH_2)_2N-$.

40. Composé selon les revendications 33, 34 ou 35 dans lequel Alk^2 et Sp^2 sont chacun une liaison.

41. Composé selon la revendication 33, dans lequel Alk^1 est une chaîne alkylène ramifiée ou non ramifiée en (C_1-C_{10}) et Sp^1 est une liaison, -S-, -O-, -CONH-, -NHCO-, -NR'-, $-N(CH_2CH_2)_2N-$ et Alk^2 et Sp^2 sont chacun une liaison.

42. Composé selon la revendication 39, dans lequel Ar est 1,2-, 1,3- ou 1,4-phénylène non substitué et Z^1 est un phényle substitué.

43. Composé selon la revendication 41, dans lequel Ar est 1,2-, 1,3- ou 1,4-phénylène non substitué et Z^1 est alkyle en (C_1-C_5).

44. Composé selon la revendication 34, dans lequel Alk^2 et Sp^2 sont chacun une liaison, Ar est 1,2-, 1,3- ou 1,4-phénylène non substitué et Z^1 est alkyle en (C_1-C_5).

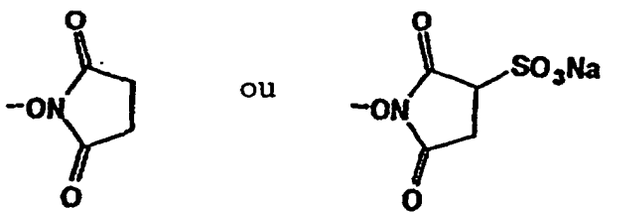
45. Composé selon la revendication 35, dans lequel Sp^1 est -S- ou $-N(CH_2CH_2)_2N-$, et Alk^2 et Sp^2 sont chacun une liaison.

46. Composé selon les revendications 39, 41 ou 45 dans lequel Ar est 1,2-, 1,3- ou 1,4-phénylène non substitué.

47. Composé selon la revendication 45, dans lequel Ar est 1,2-, 1,3- ou 1,4-phénylène non substitué et Z^1 est alkyle en (C_1-C_5).

48. Composé selon la revendication 34, dans lequel Ar est 1,2-, 1,3- ou 1,4-phénylène non substitué et Alk^2 et Sp^2 sont chacun une liaison.

49. Composé selon les revendications 38, 42, 43, 44 ou 47 dans lequel Z^3 est hydroxy,



50. Composition pharmaceutique pour inhiber la croissance de cellules, comprenant une quantité efficace inhibant la croissance cellulaire du conjugué selon la revendication 1 et un milieu qu'on peut administrer par voie parentérale.

51. Composition pharmaceutique pour inhiber la croissance de cellules, comprenant une quantité efficace inhibant la croissance cellulaire du conjugué selon la revendication 15 ou 16 et un milieu qu'on peut administrer par voie parentérale.

52. Composition pharmaceutique lyophilisée pour inhiber la croissance des cellules, comprenant un conjugué selon la revendication 15 ou 16 qui est obtenu en lyophilisant une solution approximativement de 1 mg/ml du conjugué dissous dans un tampon de phosphate de sodium d'environ 5 mM à un pH d'environ 7,4 contenant du chlorure de sodium environ 100 mM et du sucrose environ 100 mM.

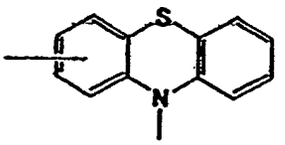
53. Procédé de préparation des dérivés ciblés de formule

$$Z^3[CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=Z^2]_m$$

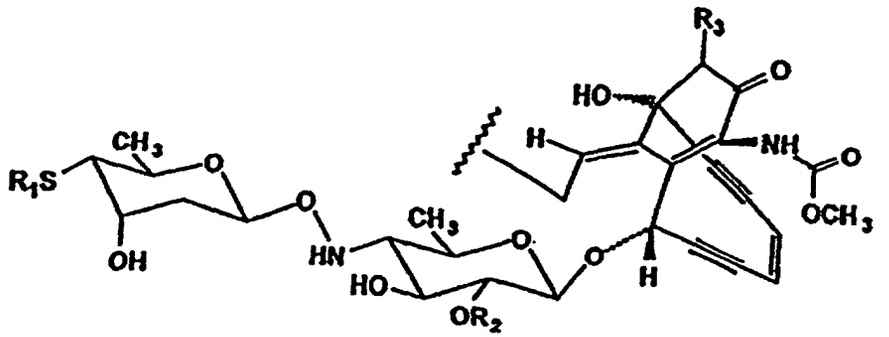
dans laquelle

Z^3 est une protéine choisie parmi les anticorps mono- et polyclonaux, leurs fragments reconnaissant un antigène, et leurs contreparties chimiquement ou génétiquement manipulées ;
Alk^1 et Alk^2 sont indépendamment une liaison ou une chaîne alkylène ramifiée ou non ramifiée en (C_1-C_{10}) ;
Sp^1 est une liaison, -S-, -O-, -CONH-, -NHCO-, -NR'-, $-N(CH_2CH_2)_2N-$ ou -X-Ar' -Y- $(CH_2)_n$-Z où X, Y, et Z sont indépendamment une liaison, -NR'-, -S- ou -O-, à condition que, quand n=0, alors au moins un des Y et Z doit être une liaison et Ar' est 1,2-, 1,3- ou 1,4-phénylène facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_5), alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, COOR', CONHR', O

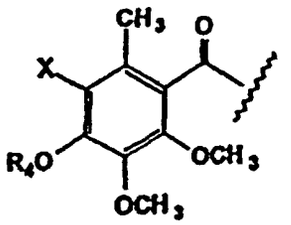
$(CH_2)_nCOOR'$, S $(CH_2)_nCOOR'$, O $(CH_2)_nCONHR'$ ou S $(CH_2)_nCONHR'$, à condition que, quand Alk^1 est une liaison, Sp^1 est aussi une liaison ;
n est un entier de 0 à 5 ;
R' est une chaîne en (C_1-C_5) ramifiée ou non ramifiée facultativement substituée par un ou deux groupements de -OH, alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, dialkylamino en (C_1-C_3) ou trialkylammonium-A^- en (C_1-C_3) où A^- est un anion pharmaceutiquement acceptable complétant un sel ;
Ar est 1,2-, 1,3- ou 1,4-phénylène, facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_6), alkoxy en (C_1-C_5), thioalkoxy en (C_1-C_4), halogène, nitro ou COOR', CONHR', $O(CH_2)_nCOOR'$, S $(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ ou $S(CH_2)_nCONHR'$ où n et R' sont comme définis ci-avant ou un 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6- ou 2,7-naphtylidène ou



chaque naphtylidène ou phénothiazine facultativement substitué avec un, deux, trois ou quatre groupements alkyle en (C_1-C_5), alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, $S(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ ou $S(CH_2)_nCOOR'$ où n et R' sont comme définis ci-avant, à condition que, quand Ar est naphtylidène, Z^1 n'est pas hydrogène et à condition que quand Ar est phénothiazine, Sp^1 est une liaison uniquement connectée à l'azote ;
Sp^2 est une liaison, -S- ou -O-, à condition que, quand Alk^2 est une liaison, Sp^2 est une liaison ;
Z^1 est H, alkyle en (C_1-C_5) ou phényle facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_5), alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, COOR', CONHR', $O(CH_2)_nCOOR'$, S $(CH_2)_nCOOR'$, $O(CH_2)_nCONHR'$ ou $S(CH_2)_nCONHR'$ où n et R' sont comme définis ci-avant ;
Z^2 est Q-Sp-S-S-W, où W est

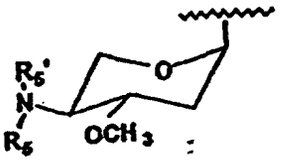


R_1 est

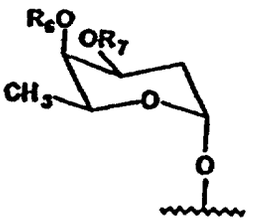


ou CH_3 ;

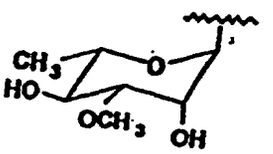
R_2 est



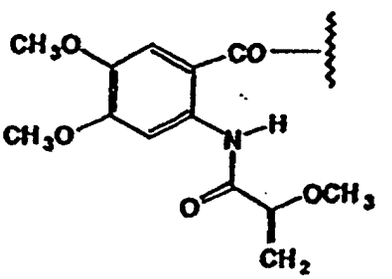
ou H ;
R_3 est



ou H ;
R_4 est



ou H ;
R_6 ou R_7 est H ou



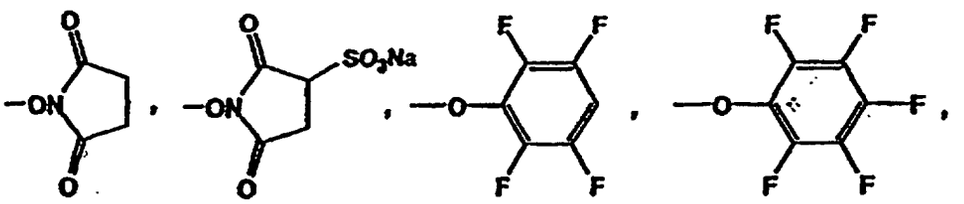
R_5 est $-CH_3$, $-C_2H_5$ ou $-CH(CH_3)_2$; X est un atome d'iode ou de brome ; R_5' est un hydrogène ou le groupement RCO où R est hydrogène, groupement alkyle en (C_1-C_{10}) ou alkylène en (C_1-C_{10}) ramifié ou non ramifié, un groupement aryle en (C_6-C_{11}), un groupement aryl(C_6-C_{11})-alkyle(C_1-C_5) ou un groupement hétéroaryle ou hétéroaryl-alkyle(C_1-C_5) dans lequel l'hétéroaryle est défini comme 2- ou 3-furyle, 2-ou 3-thiényle, 2- ou 3-(N-méthylpyrrolyle), 2-, 3- ou 4-pyridyle, 2-, 4- ou 5-(N-méthylimidazolyle), 2-, 4- ou 5-oxazolyle, 2-, 3-, 5- ou

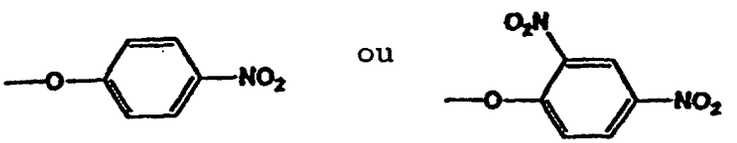
6-pyrimidinyle, 2-, 3-, 4-, 5-, 6-, 7- ou 8-quinolyle, ou 1-, 3-, 4-, 5-, 6-, 7- ou 8-isoquinolyle, tous les groupements aryles et hétéroaryles étant facultativement substitués par un ou plusieurs groupements hydroxy, amino, carboxy, halogène, nitro, alkoxy inférieur en (C_1-C_3) ou thioalkoxy inférieur en (C_1-C_5) ;
Sp est un radical divalent ou trivalent à chaîne droite ou ramifiée en (C_1-C_{18}), un radical divalent ou trivalent aryle ou hétéroaryle, un radical divalent ou trivalent cycloalkyle en (C_3-C_{18}) ou hétérocycloalkyle, un radical divalent ou trivalent aryl- ou hétéroaryl-alkyle en (C_1-C_{18}), un radical divalent ou trivalent cycloalkyl- ou hétérocycloalkyl-alkyle en (C_1-C_{18}), ou un radical divalent ou trivalent alkyle insaturé en (C_2-C_{18}), où l'hétéroaryle est furyle, thiényle, N-méthylpyrrolyle, pyridinyle, N-méthylimidazolyle, oxazolyle, pyrimidinyle, quinolyle, isoquinolyle, N-méthylcarbazoyle, aminocoumarinyle ou phénazinyle et où si Sp est un radical trivalent, il peut être en plus substitué par un groupement dialkylamino inférieur en (C_1-C_5), alkoxy inférieur en (C_1-C_5), hydroxy ou alkylkthio inférieur en (C_1-C_5) ;
Q est =NHNCO-, =NHNCS-, =NHNCONH-, =NHNCSNH- ou =NO- ; et
m est d'environ 0,1 à 15 ;

comprenant la réaction d'un composé de formule

$$Z^{3'}CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C(Z^1)=Z^2$$

dans laquelle Alk^1, Sp^1, Ar, Alk^2, Z^1 et Z^2 sont comme définis ci-dessus ; et $Z^{3'}$ est





avec un support Z^3, où Z^3 est une protéine choisie parmi les anticorps mono- et polyclonaux, leurs fragments reconnaissants un antigène et leurs contreparties chimiquement ou génétiquement manipulées, dans une solution aqueuse tamponnée à un pH entre 6,5 et 9,0 et une température de 4°C à 40°C pendant 1 à 48 heures pour générer les dérivés ciblés de formule

$$Z^3\ [CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C\ (Z^1)\ =Z^2]_m$$

définie ci-dessus.

54. Procédé selon la revendication 53, dans lequel le composé de formule

$$Z^{3'}CO\text{-}Alk^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C\ (Z^1)\ =Z^2$$

est généré par :

(a) réaction de H_2Z^2 avec un composé de formule

$$HOCO\text{-}Akl^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C\ (Z^1) =O$$

dans un solvant alcoolique avec un point d'ébullition de moins d'environ 100°C en présence d'acide acétique à environ 5% ou d'un catalyseur d'acide carboxylique à environ 20°C à 70°C pendant environ 1 à 24 heures, où Alk^1 et Alk^2, Sp^1, n, R', Sp^2, Z^1 et Ar sont comme définis dans la revendication 53 pour produire un intermédiaire de formule

$$HOCO\text{-}Akl^1\text{-}Sp^1\text{-}Ar\text{-}Sp^2\text{-}Alk^2\text{-}C\ (Z^1)=Z^2,$$

dans laquelle Alk^1, Sp^1, Ar, Sp^2, Alk^2, Z^1 et Z^2 sont comme définis dans la revendication 53 ;
(b) isolement de l'intermédiaire de l'étape (a) ; et
(c) réaction de l'intermédiaire isolé de l'étape (b) avec N-hydroxysuccinimide, 2,3,5,6-tétrafluorophénol, pentafluorophénol, 4-nitrophénol, 2,4-dinitrophénol ou N-hydroxysulfosuccinimide en présence de DCC, EDCI ou autre carbodiimide dans un solvant organique inerte comme l'acétonitrile

ou l'acétonitrile contenant 5-50% DMF.

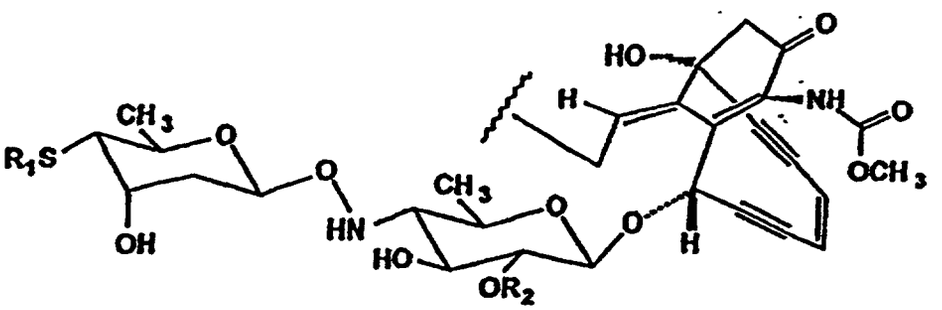
55. Procédé selon la revendication 53 ou 54, dans lequel Alk^2 et Sp^2sont conjointement une liaison et Z^1 est H ou alkyle en (C_1-C_5).

56. Procédé selon la revendication 54 ou 55, dans lequel Sp^1 est une liaison, -S-, -O-, -CONH-, -NHCO- ou -NR', à condition que, quand Sp^1 est une liaison, Alk^1 est une liaison.

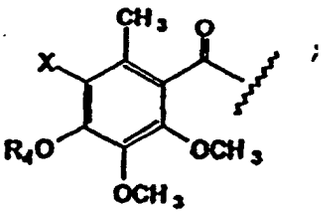
57. Procédé selon la revendication 54 ou 55, dans lequel Ar est 1,2-, 1,3- ou 1,4-phénylène facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_6), alkoxy en (C_1-C_5), thioalkoxy en (C_1-C_4), halogène, nitro, COOR', CONHR', O $(CH_2)_n$COOR', S $(CH_2)_n$COOR', O $(CH_2)_n$CONHR' ou S $(CH_2)_n$CONHR' ou un 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6- ou 2,7-naphtylidène, chacun facultativement substitué avec un, deux, trois ou quatre groupements alkyle en (C_1-C_6), alkoxy en (C_1-C_4), thioalkoxy en (C_1-C_4), halogène, nitro, COOR', CONHR', O $(CH_2)_n$COOR', S $(CH_2)_n$COOR', O $(CH_2)_n$CONHR' ou S $(CH_2)_n$CONHR'.

58. Procédé selon la revendication 54 ou 55, dans lequel une liaison covalente à la protéine Z^3 est un amide formé à partir d'une réaction avec les chaînes latérales de lysine de la protéine Z^3.

59. Procédé selon la revendication 54 ou 55, dans lequel Z^2 est Q-Sp-S-S-W et W est

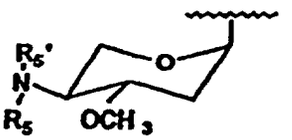


R_1 est



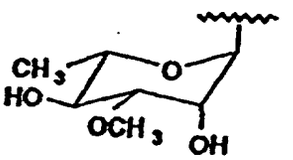
;

R_2 est



ou H ;

R_4 est



ou H ;

R_5, X, $R_{5'}$, R, et Sp sont comme définis dans la revendication 49 et Q est =NHNCO-.

60. Procédé selon la revendication 54, dans lequel le solvant alcoolique de l'étape (a) est le méthanol ; le catalyseur d'acide carboxylique de l'étape (a) est de l'acide acétique à 5% ; l'intermédiaire isolé de l'étape (b) est mis à réagir à l'étape (c) avec du N-hydroxy-succinimide en présence de EDCI dans de l'acétonitrile ; et la solution aqueuse tamponnée de l'étape (d) est un tampon phosphate ayant un pH de 7,4 à 8,0.

61. Procédé selon la revendication 60, dans lequel Z^1 est alkyle en (C_1-C_5).

62. Procédé selon la revendication 61, dans lequel Ar est 1,2-, 1,3- ou 1,4-phénylène facultativement substitué avec un, deux ou trois groupements alkyle en (C_1-C_6), alkoxy en (C_1-C_5), thioalkoxy en (C_1-C_4), halogène, nitro ou COOR', CONHR', O $(CH_2)_n$COOR', S $(CH_2)_n$COOR', O$(CH_2)_n$CONHR' ou S $(CH_2)_n$CONHR'.

63. Procédé selon la revendication 62, dans lequel Sp^1 est -O-, Alk^1 est alkylène en C_4, Ar est 1,4-phénylène et Z^1 est alkyle en C_1.

64. Procédé selon la revendication 63, dans lequel Z^3 est l'anticorps h-P67.6, h-CT-M-01, m-CT-M-01, h-A33, m-A33 ou anti-Tac.

65. Procédé selon la revendication 64, dans lequel Z^2 est le calichéamicine gamma diméthylhydrazide ou calichéamicine N-acétyl gamma diméthylhydrazide.

66. Procédé selon la revendication 65, dans lequel Z^3 est l'anticorps h-CT-M-01 et Z^2 est le calichéamicine N-acétyl gamma diméthylhydrazide.

67. Procédé selon la revendication 65, dans lequel Z^3 est l'anticorps h-P67.6 et Z^2 est le calichéamicine N-acétyl gamma diméthylhydrazide.

68. Procédé selon la revendication 57, dans lequel Sp^1 est -O- ou une liaison ; Alk^1 est une liaison ou une chaîne alkylène ramifiée ou non ramifiée en (C_1-C_{10}), à condition que quand Alk^1 est une liaison, Sp^1 est une liaison ; Z^1 est alkyle en (C_1-C_5) ; et Z^2 est le calichéamicine N-acétyl gamma diméthylhydrazide.

69. Conjugué selon l'une quelconque des revendications 1 à 18, pour l'utilisation pour contrôler la croissance d'une cellule indésirable chez un mammifère.

70. Composé pour l'utilisation selon la revendication 69, dans lequel la cellule indésirable est une cellule cancéreuse.

71. Composé selon la revendication 70 dans lequel le cancer est le cancer mammaire, pulmonaire ou ovarien ou la leucémie.

72. Utilisation d'un conjugué selon l'une quelconque des revendications 1 à 18, dans la fabrication d'un médicament pour contrôler la croissance d'une cellule indésirable chez un mammifère.

73. Utilisation selon la revendication 72, dans laquelle la cellule indésirable est une cellule cancéreuse.

74. Utilisation selon la revendication 73 dans laquelle le cancer est le cancer mammaire, pulmonaire ou ovarien ou la leucémie.

Chart 1: LIGHT CHAIN FOR h-P67

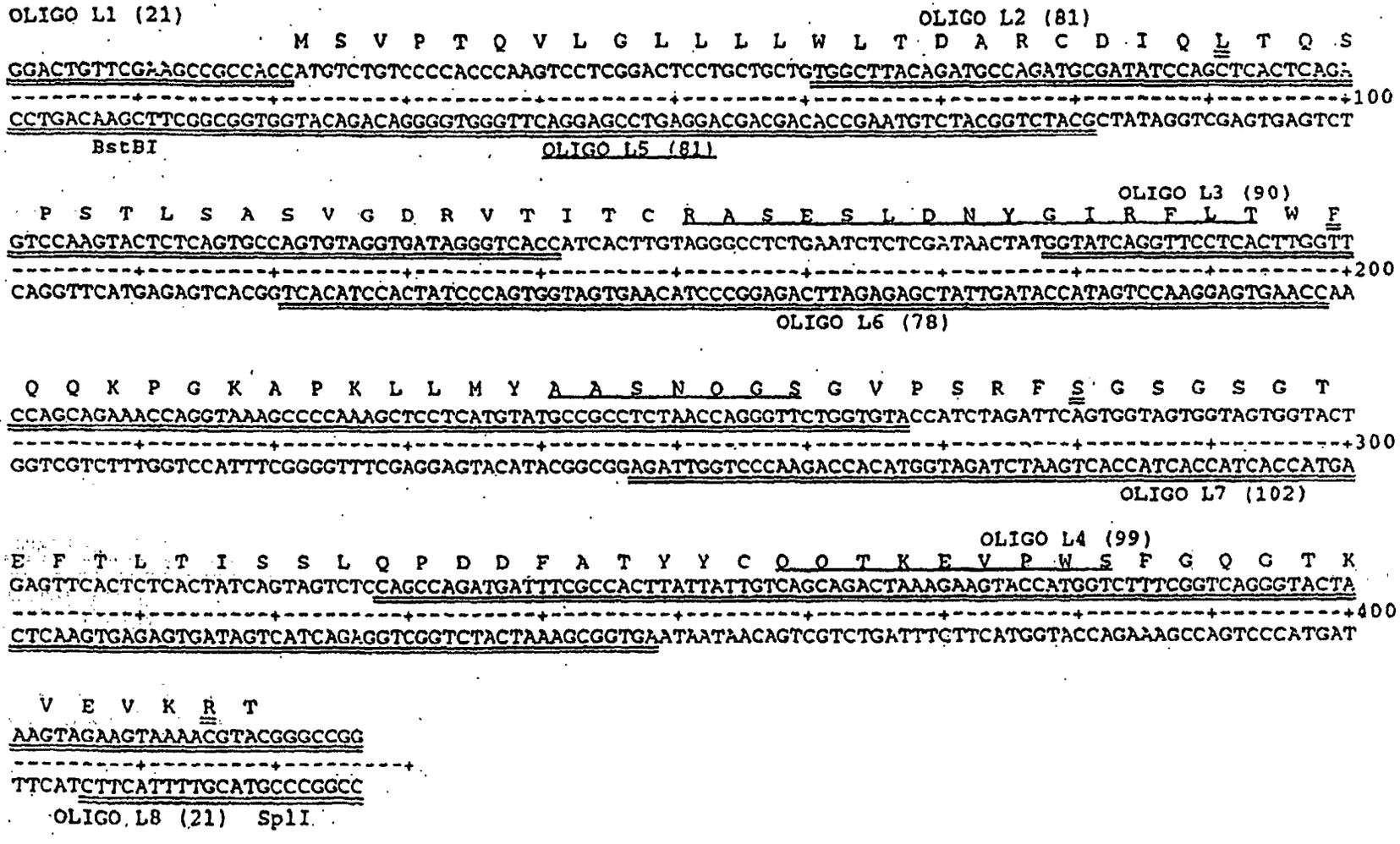


Chart 2: HEAVY CHAIN FOR h-P67

OLIGO H1 (21)

```
                    M  E  W  S  W  V  F  L  F  F  L  S  V  T  T  G  V  H  S  E  V  Q  L  V  Q  S  G
GCGCGCAAGCTTGCCGCCACCATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACTACAGGAGTCCATTCTGAGGTGCAGCTGGTGCAGTCTG
---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+
CGCGCGTTCGAACGGCGGTGGTACCTTACCTCGACCCAGAAAGAGAAGAAGGACAGTCATTGATGTCCTCAGGTAAGACTCCACGTCGACCACGTCAGAC  100
     Hind III                                                  OLIGO H5 (96)
```

OLIGO H2 (96)

```
  A  E  V  K  K  P  G  S  S  V  K  V  S  C  K  A  S  G  Y  T  I  T  D  S  N  I  H  W  V  R  Q  A  P
GAGCAGAGGTGAAGAAGCCTGGATCTTCTGTGAAGGTGTCTTGTAAGGCATCTGGATACACAATTACAGACTCCAATATTCACTGGGTGAGACAGGCACC
---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+
CTCGTCTCCACTTCTTCGGACCTAGAAGACACTTCCACAGAACATTCCGTAGACCTATGTGTTAATGTCTGAGGTTATAAGTGACCCACTCTGTCCGTGG  200
                                                                OLIGO H6 (96)
```

OLIGO H3 (96)

```
  G  Q  S  L  E  W  I  G  Y  I  Y  P  Y  N  G  G  T  D  Y  N  Q  K  F  K  N  R  A  T  L  T  V  D  N
TGGACAGTCCCTCGAGTGGATTGGATACATTTACCCTTACAATGGAGGAACAGACTACAATCAGAAGTTCAAGAATAGAGCAACACTGACAGTGGACAAT
---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+
ACCTGTCAGGGAGCTCACCTAACCTATGTAAATGGGAATGTTACCTCCTTGTCTGATGTTAGTCTTCAAGTTCTTATCTCGTTGTGACTGTCACCTGTTA  300

  P  T  N  T  A  Y  M  E  L  S  S  L  R  S  E  D  T  A  F  Y  Y  C  V  N  G  N  P  W  L  A  Y  W  G  Q
CCTACGAATACCGCCTACATGGAGCTGTCTTCTCTGAGATCTGAGGACACAGCATTCTACTACTGTGTGAATGGAAATCCTTGGCTGGCTTACTGGGGAC
---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+
GGATGCTTATGGCGGATGTACCTCGACAGAAGAGACTCTAGACTCCTGTGTCGTAAGATGATGACACACTTACCTTTAGGAACCGACCGAATGACCCCTG  400
                                  OLIGO H7 (84)
```

OLIGO H4 (86)

```
  G  T  L  V  T  V  S  S  A  S  T  K  G  P
AGGGAACACTGGTGACAGTGTCTTCTGCCTCAACGAAGGGCCCGCGCGC
---------+---------+---------+---------+---------+ 450
TCCCTTGTGACCACTGTCACAGAAGACGGAGTTGCTTCCCGGGCGCGCG
                    OLIGO H8 (21) ApaI
```

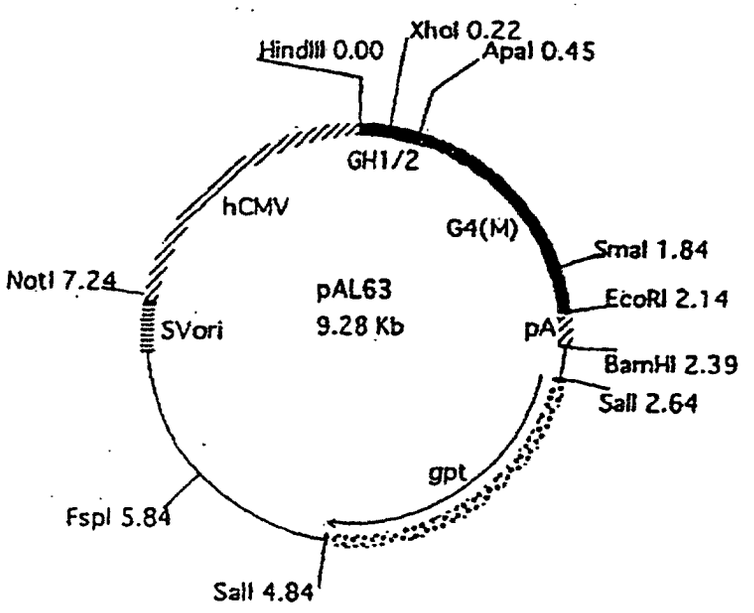

HindIII 0.00
XhoI 0.22
ApaI 0.45
GH1/2
hCMV
G4(M)
SmaI 1.84
NotI 7.24
pAL63
9.28 Kb
EcoRI 2.14
SVori
pA
BamHI 2.39
SalI 2.64
gpt
FspI 5.84
SalI 4.84

Chart 3

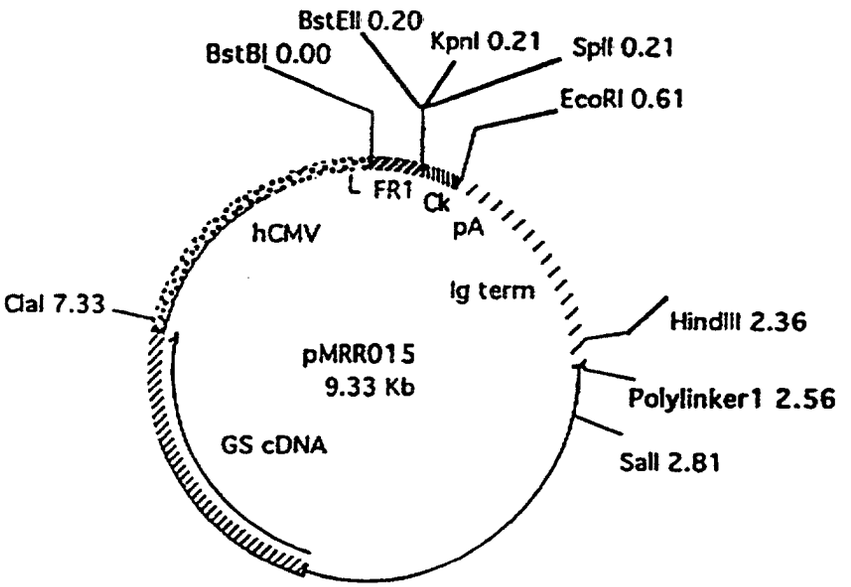

BstEII 0.20
KpnI 0.21
SpII 0.21
BstBI 0.00
EcoRI 0.61
L
FR1
Ck
hCMV
pA
Ig term
ClaI 7.33
HindIII 2.36
pMRR015
9.33 Kb
Polylinker1 2.56
GS cDNA
SalI 2.81

Chart 4

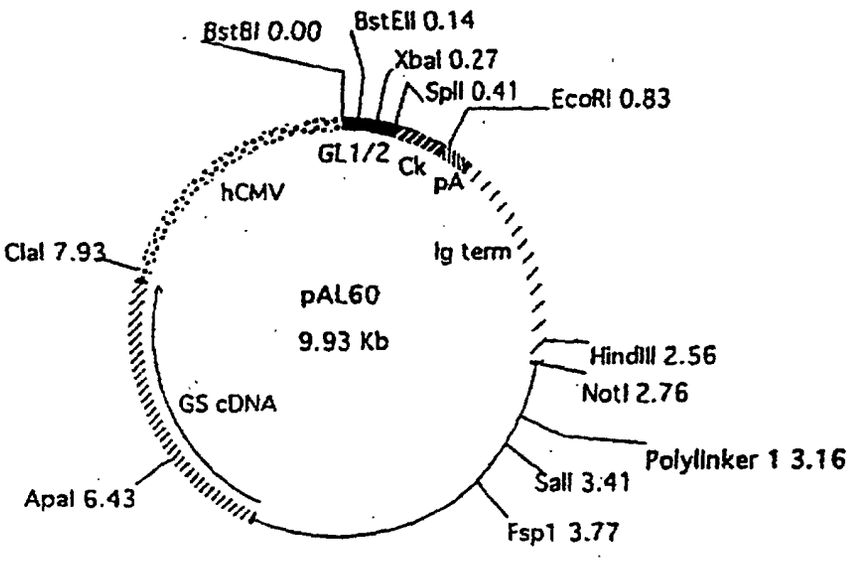

BstBI 0.00
BstEII 0.14
XbaI 0.27
SpII 0.41
EcoRI 0.83
GL1/2
Ck
pA
hCMV
ClaI 7.93
Ig term
pAL60
9.93 Kb
HindIII 2.56
NotI 2.76
GS cDNA
PolyIInker 1 3.16
SalI 3.41
ApaI 6.43
Fsp1 3.77

Chart 5

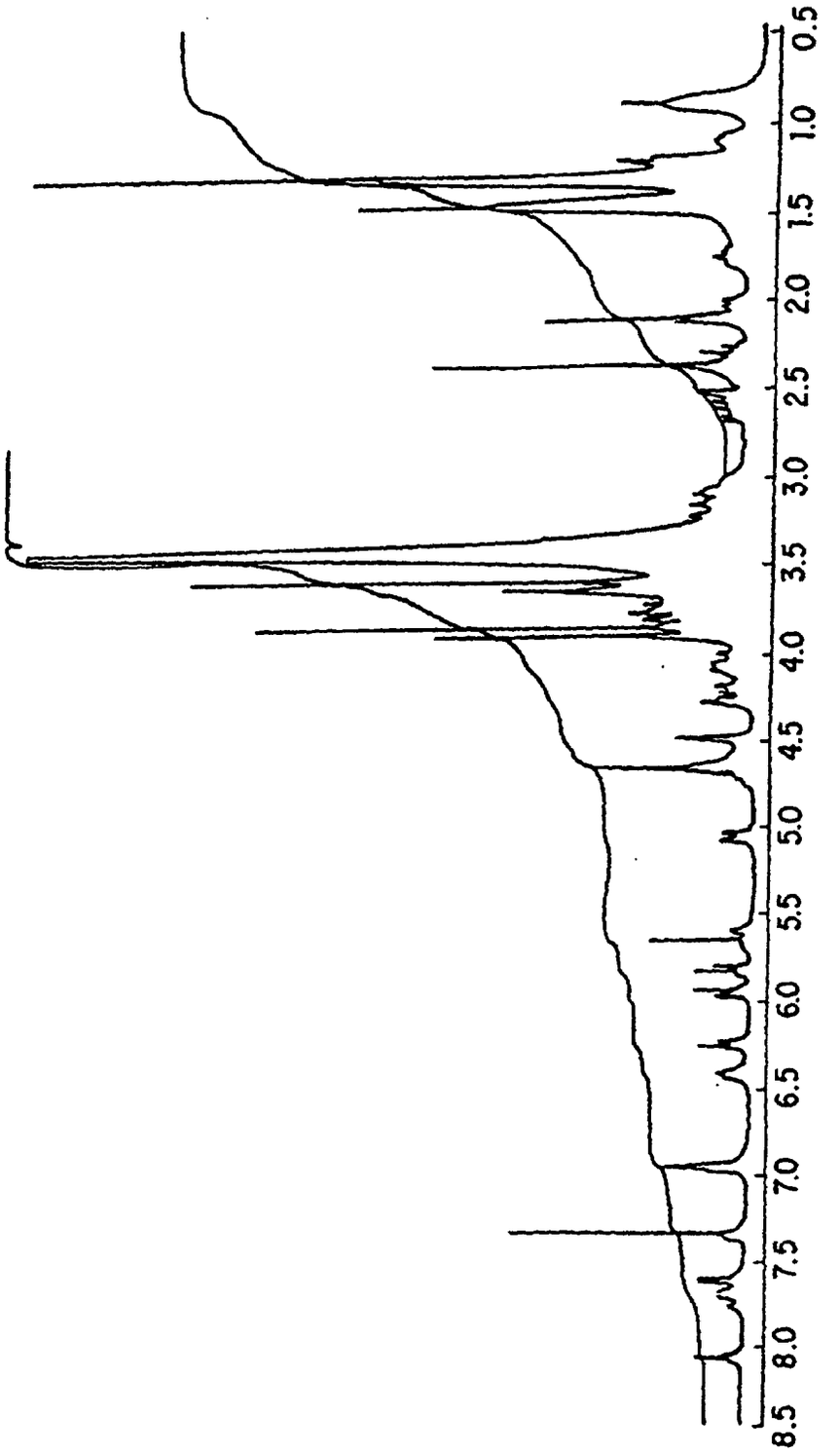


FIG 1

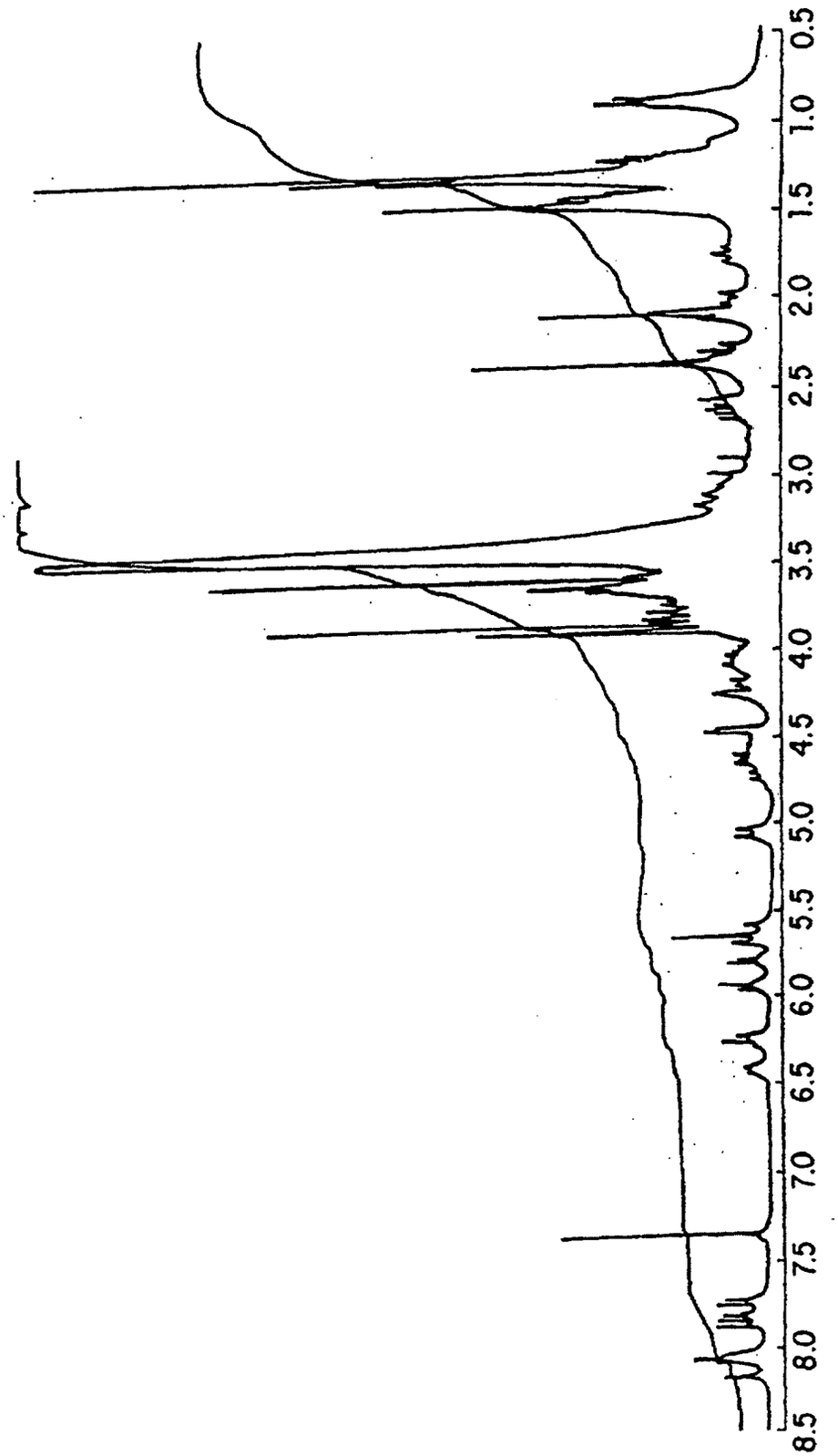


FIG 2

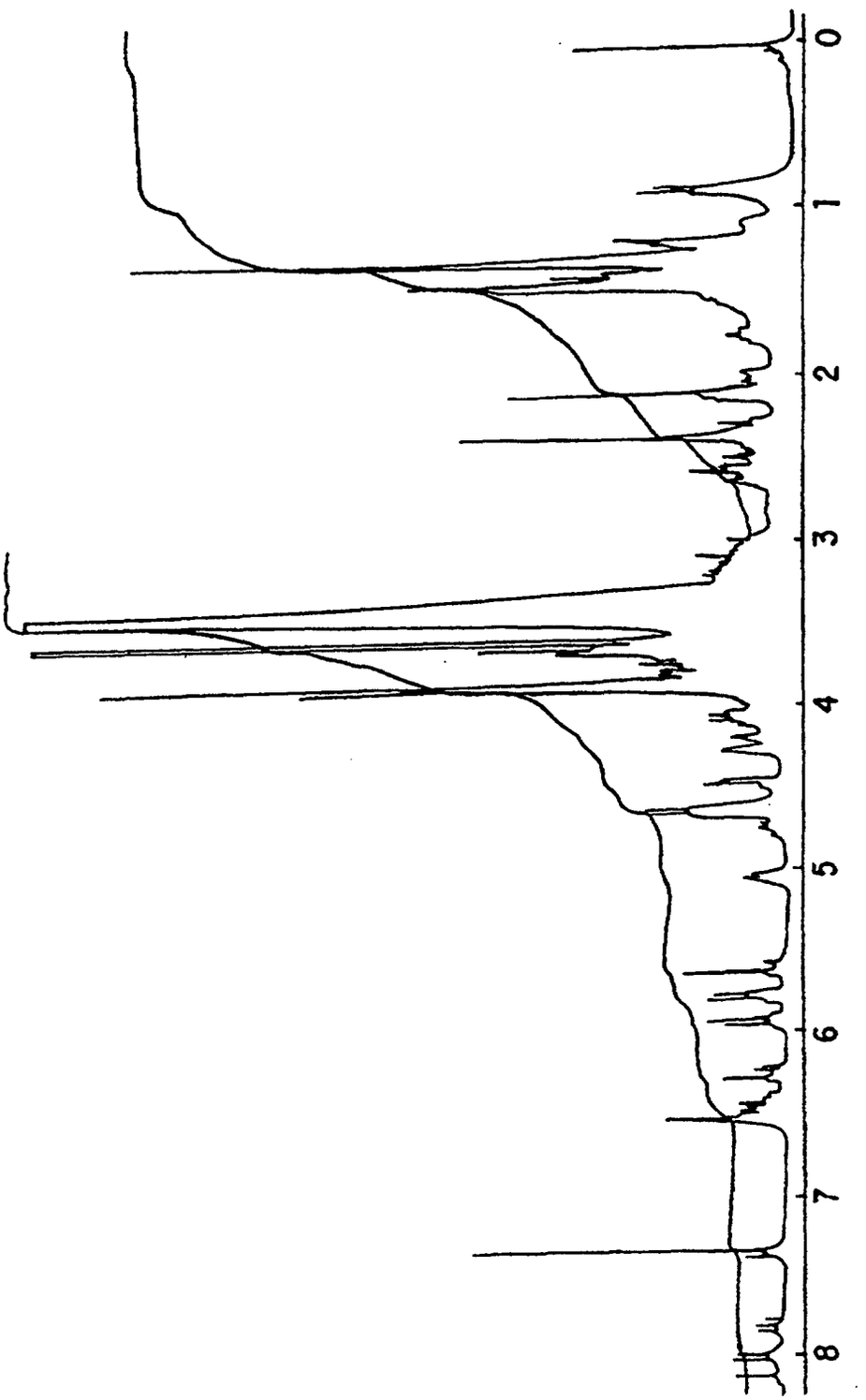


FIG 3

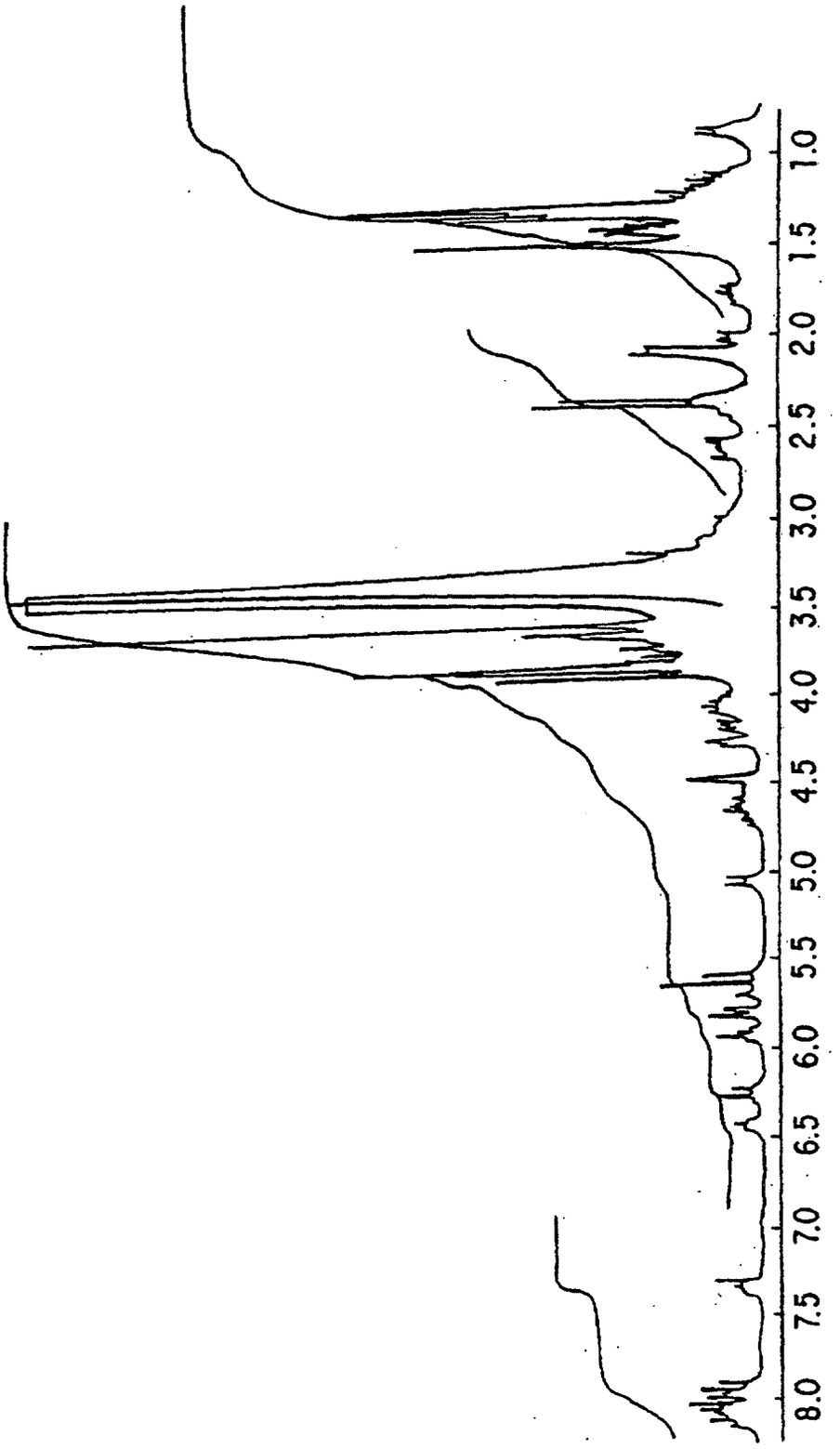


FIG 4

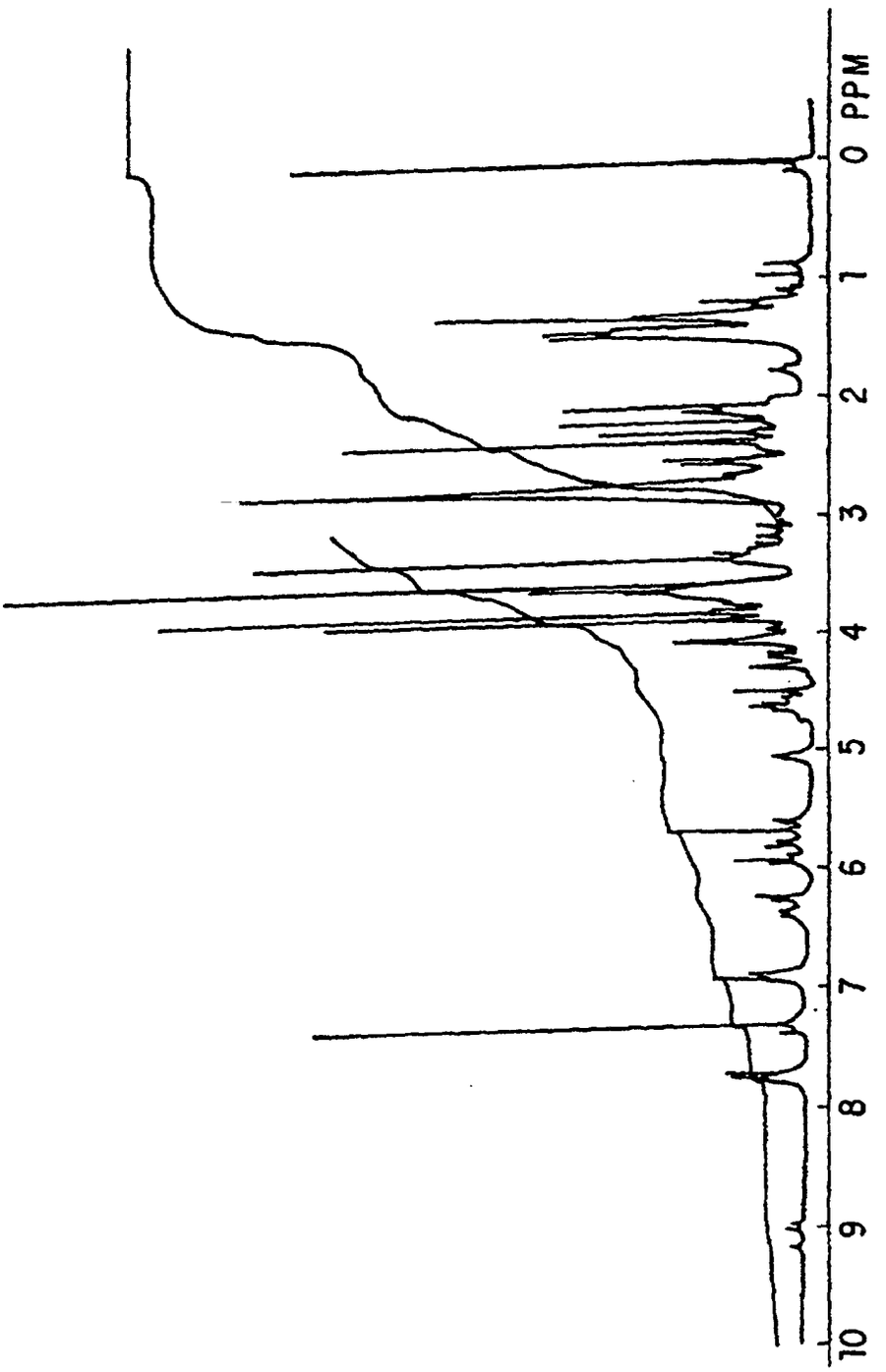


FIG 5

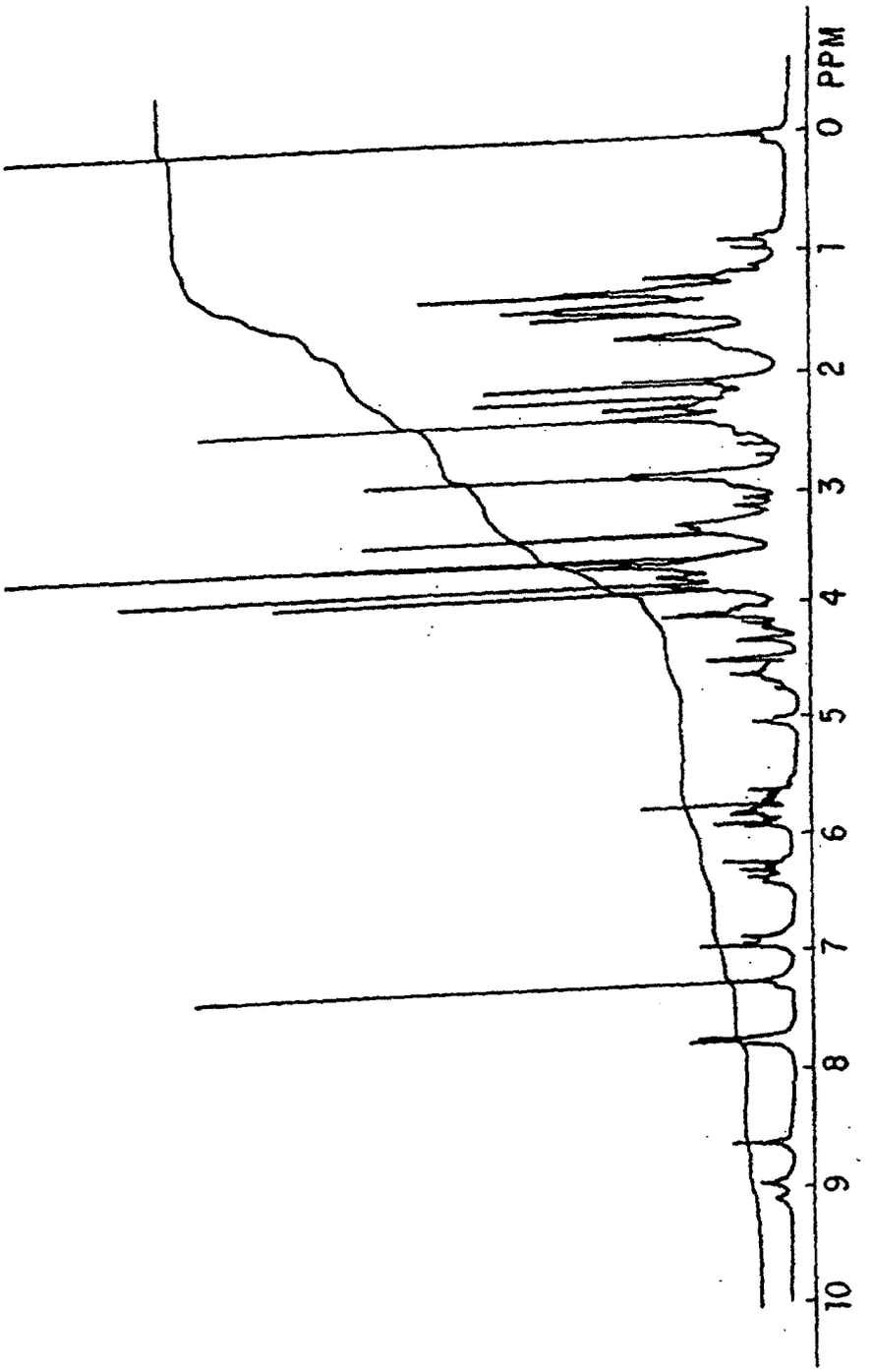


FIG 6

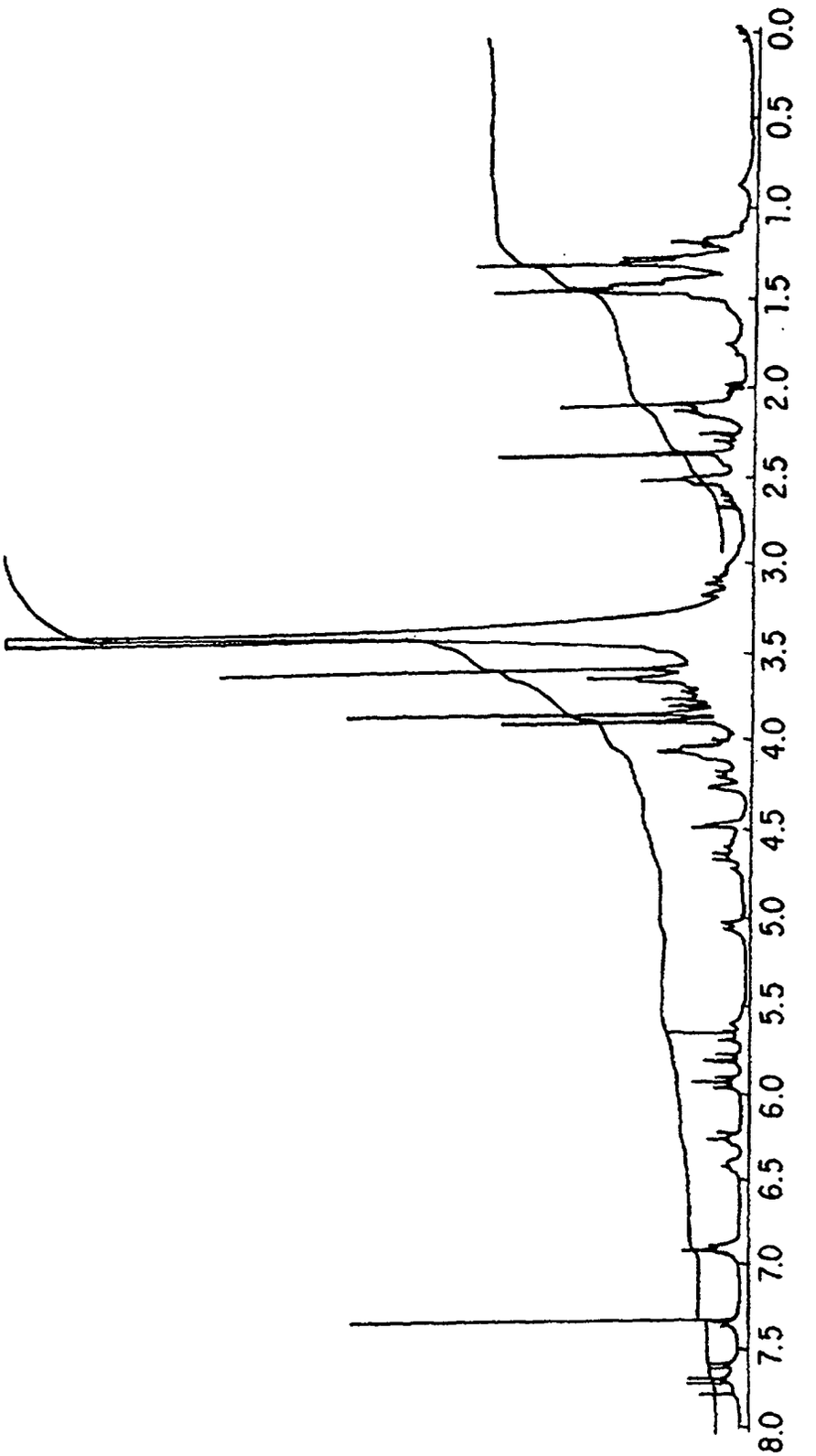


FIG 7